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Modifications of Current Properties by Expression of a Foreign Potassium Channel Gene in *Xenopus* Embryonic Cells

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Abstract. The development of excitable cells is characterized by highly organized patterns of expression of ion channels. During the terminal differentiation of *Xenopus* muscle somites, potassium currents are expressed first just after Stage 15 (early-mid neurula), following a long period during which no voltage-dependent currents can be detected in any cell in the dorsal embryo. We have investigated whether early expression of a foreign delayed rectifier potassium channel may affect this endogenous pattern of electrical development. We injected the purified cRNA of the mammalian brain Shaker-like potassium channel, Kv1.1, into fertilized *Xenopus* eggs. The resulting currents were analyzed in blastomeres during a 12-hr period prior to Stage 15 and in differentiating muscle cells after Stage 15. In injected embryos, a high fraction of blastomeres expressed a delayed rectifier-type current. The Kv1.1 current could be distinguished from the endogenous muscle delayed potassium current ($I_{K,X}$) by its very different voltage dependence. Separation of currents based on this difference indicated that, in injected embryos, $I_{K,X}$ appeared much earlier in development than in control embryos. Furthermore, even in cells which expressed solely Kv1.1-type current, the sensitivity of the current to dendrotoxin declined dramatically during development, approaching that of $I_{K,X}$. These data suggest an interaction between Kv1.1 and endogenous channel subunits, and/or modification of the Kv1.1 protein by the embryonic cells in ways not seen in *Xenopus* oocytes or mammalian cell lines.

Key words: K channel — Heterologous expression — Electrical development — Muscle — Patch clamp — Embryo — *Xenopus laevis.*

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Introduction

The expression of ion channels is closely regulated during development and their function early in embryogenesis influences later development. For example, in the developing mammalian visual system, the refinement of connections between the retina and central structures is dependent on spontaneous electrical activity that occurs before the development of light-stimulated pathways (Meister et al., 1991; Wong, Meister & Shatz, 1993). Electrical activity can also regulate development of isolated embryonic cells. Influx of calcium through calcium channels during spontaneous electrical activity of amphibian neurons is required to promote differentiation of a delayed potassium current (Desarmenien & Spitzer, 1991) as well as neurite outgrowth and the acquisition of neurotransmitter phenotype (Holliday et al., 1991). Further, neuronal differentiation is adversely affected by the creation of abnormal patterns of expression of ion channels (Jones & Ribera, 1994). Muscle phenotype, in particular the expression of ion channels, is also under the control of electrical activity. For instance, electrical activity regulates the expression of sodium channels during later stages of differentiation (Sherman & Caterall, 1984; Offord & Caterall, 1989) as well as the normal expression levels of acetylcholine receptors (Lomo & Westgaard, 1975; Heathcote, 1989) and their subunits (Dutton, Simon & Burdon, 1993).

We have previously described the sequential appearance of ionic currents in differentiating *Xenopus* myocytes isolated from Stage 15 embryos (Spruce & Moody, 1992). The present study further emphasizes that all blastomeres (presumably including muscle precursors) isolated prior to Stage 15 and after the onset of gastrula, contained no voltage-dependent currents. Investigation of the significance of this pattern of electrical differentiation is perhaps best achieved by specific disruption of

the normal developmental profiles of ion channel expression. In this study, we attempt to create such a disruption in embryonic *Xenopus* cells by injecting cRNA for the mammalian Shaker-type channel, Kv1.1, into fertilized *Xenopus* eggs. Although the oocyte has been employed extensively as a heterologous expression system for ion channels, the embryo has only rarely been so used (Spruce, Hopkins & Moody, 1993; Jones & Ribera, 1994; Honore et al., 1994; Linsdell & Moody, 1994). We describe the appearance of outward potassium current in cells of the gastrula and neurula stages of injected embryos, up to 12 hr before endogenous potassium currents appear in control embryos. The Kv1.1 current expressed in *Xenopus* oocytes differed in three major ways from Kv1.1 expressed in oocytes. First, the current showed substantial inactivation in embryonic cells, whereas in *Xenopus* oocytes it does not (Hopkins, Demas & Tempel, 1994). Second, as development progressed, the DTX sensitivity of the current declined gradually to levels much lower than that of Kv1.1 expressed in oocytes (Hopkins et al., 1994). Third, the voltage dependence of the current changed during development. This last effect could be explained if Kv1.1 expression accelerated the development of endogenous delayed potassium current so that it appeared up to 6 hr earlier than normal, and was upregulated at later times. Consistent with this interpretation is our finding that the endogenous inwardly rectifying potassium current was upregulated, in addition. A brief account of some of these results has been presented previously (Spruce et al., 1993).

Materials and Methods

Preparation

Xenopus laevis embryos were generated as described previously (Spruce & Moody, 1992). Briefly, mature oocytes were extruded into 1× MBS from females injected with 1,000 i.u. human chorionic gonadotrophin and fertilized with sperm solution (in 1× MBS) prepared from an excised testis. After one-half hour, the jelly coat was removed using 2% cysteine solution. Embryos were rinsed in 0.1× MBS and then left in this solution to develop.

Dorsal tissue was excised from the embryos at different stages from early gastrula through early/mid neurula. At gastrula stages, the entire tissue fragment (between about one-quarter and one-eighth of the embryo) was incubated in Ca/Mg-free solution for up to one-half hour. The dissociated cells were then plated on to tissue culture plastic (Falcon 3001) in Danilchik's medium. At neurula stages, the dissection procedure was more complicated to enable isolation of tissue which will become the muscle somites. The neural plate region of the embryo (including underlying mesodermal tissue) was excised. The tissue was initially placed into 1× MBS solution containing 1 mg/ml papain. After 10 min, the different tissue types were easily separated and the mesodermal tissue on either side of the notocord was removed and placed into Ca/Mg-free solution for 20 min. The dissociated cells, which are mainly presumptive muscle cells, were again plated on to tissue culture plastic in Danilchik's medium.

Recordings were generally begun 1 hr after dissociation and timed relative to Stage 15 (T = 0 hr; Nieuwkoop & Faber, 1967; an early/mid neurula stage—2 hr before segregation of first myotome, 9 hr before muscle can be stimulated to contract and 17.5 hr after fertilization). The time spent in culture was included in the calculation of the timing of a recording.

RNA Injection

The expression patterns of embryonic currents in developing blastomeres were disrupted by forcing expression of a mouse brain potassium channel, Kv1.1 (Tempel, Jan & Jan, 1988; a gift from Prof. B.L. Tempel, VA Medical Center, Seattle, WA). Single-cell embryos were transferred to 1× MBS and injected with 30 nl of 5–100 pg/nl Kv1.1 cRNA using a picospritzer (WPI, Sarasota, FL). After injection, the embryos were returned to 0.1× MBS. The injected embryos appeared to develop normally. Dye was not coinjected and the presence of Kv1.1 cRNA in cells isolated from developing embryos was only assessed functionally. Not all cells contained exogenous current indicating mosaic expression which is to be expected if cRNA is compartmentalized by cell division.

Solutions

1× MBS (Gurdon, 1977) contained (in mM): 88 NaCl, 1 KCl, 2.4 $NaHCO_3$, 0.82 $MgSO_4$, 0.33 $Ca(NO_3)_2$, 0.41 $CaCl_2$, 10 Hepes, pH 7.4 (with NaOH). Cysteine solution contained 2% cysteine titrated with NaOH to pH 7.8. Danilchik's medium (Keller et al., 1985) contained (in mM): 53 NaCl, 15 $NaHCO_3$, 4.5 K · gluconate, 1 $MgSO_4$, 1 $CaCl_2$, 27 Na · isethionate, pH 8.3 (with Na_2CO_3). The external recording solution which was usually applied to the cell via a spritzing electrode, contained (in mM): 120 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 Hepes, pH 7.4 (with NaOH). For some experiments, the I fraction of Dendrotoxin (DTX-I, a potassium channel blocker) was added to this solution (A gift from Prof. B.L. Tempel, VA Medical Center, Seattle, WA; *see* Hopkins et al. (1994) for details of their procedure for purifying the I-fraction). The intracellular (pipette) solution contained (in mM): 80 K · aspartate, 10 KCl, 10 NaCl, 2 $MgCl_2$, 2 EGTA, 3 glucose, 2 theophylline, 2 Na_2ATP, 0.1 cAMP, 10 Hepes, pH 7.4 (with KOH).

Electrical Recording

The methods are described in detail elsewhere (Spruce & Moody, 1992). Whole-cell patch clamp recordings were made from the dissociated embryonic cells. Usually, the seal was made in Danilchik's medium and then external recording solution was spritzed on to the cell via an electrode placed to within 100 μm. In some experiments, the effect of DTX-I was assessed by exchanging Danilchik's medium for external recording solution in the bath and then either spritzing external recording solution containing DTX-I on to the cell or exchanging the entire bath solution for one containing DTX-I.

Currents were measured using a List Electronic EPC-7 amplifier (Darmstadt, Germany), filtered at 1 kHz (8 pole Bessel filter), acquired at 2 or 10 kHz using PCLAMP software (Axon Instruments, Foster City, CA). The scaled leak current (measured in response to 10-mV steps between −38 and −58 mV) was subtracted from all traces. A quantitative analysis of the expression of outward potassium currents was made by measuring the current at +22 mV (after correction for liquid junction potential) and at 155 msec after the voltage step. The size of the inward rectifier potassium current was measured as the maximum current 750–800 msec after the voltage step. Current density was measured by dividing total cell current by cell capacitance. The degree of inactivation of outward potassium current was measured at +32 mV after digital subtraction of an appropriately scaled fit to the

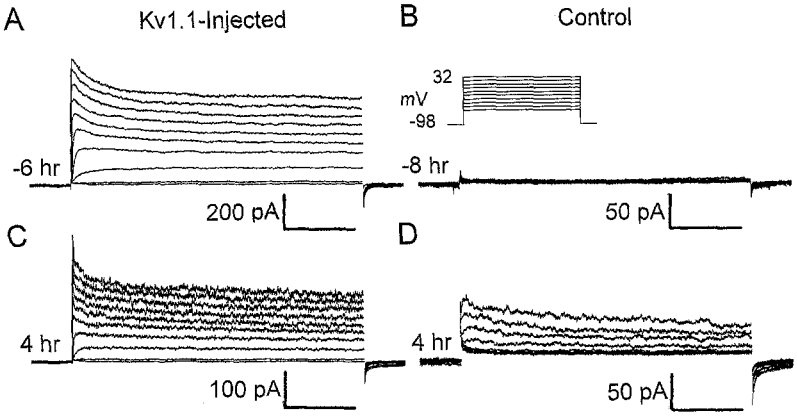

Fig. 1. Potassium current expression following injection of Kv1.1 cRNA. (*A*) and (*C*) Both sets of current traces are recordings from different cells (6 hr before (*A*) and 4 hr after (*C*) Stage 15), isolated from embryos which had been injected with Kv1.1 cRNA at the 1-cell stage. The cells were held at −98 mV and step depolarizations were applied at 10 mV increments for 800 msec between −58 and +32 mV (schematic of voltage protocol in *B*). External recording solution was either present in the bath (*A*) or spritzed on to the cell (*C*). (*B*) and (*D*) Again, the current traces are recordings from different cells (8 hr before (*B*) and 4 hr after (*D*) Stage 15), but now they had been isolated from control embryos (*see* Results for definition of control). Time scale is 100 msec for all traces.

capacity currents at −58 mV. Fractional inactivation (FI) was calculated according to the following equation:

$$FI = (I_{pk} - I_{SS})/I_{pk},$$

where I_{pk} is peak potassium current amplitude within 60 msec from beginning of the pulse, averaged over 5 msec, and I_{SS} is the amplitude after 155 msec, averaged over 10 msec.

In cells from injected embryos, the separate expression patterns of endogenous outward potassium current ($I_{K,X}$) and the potassium current resulting from Kv1.1 expression ($I_{K,I}$) were determined using a method which relied on their differing voltage dependencies of activation (*see* Results). An important assumption of this analysis is that the voltage-dependence of Kv1.1 current is unaffected by embryonic expression. This will be discussed later.

All potentials were corrected for the liquid junction potential. Between K · asp and either Danilchik's medium or external recording solution, it was −8 and −9 mV, respectively. Means are expressed as ±SE unless otherwise specified.

Results

Expression of a K^+ Current in Embryonic Cells after Injection of cRNA

Voltage-dependent currents appear in differentiating nerve and muscle cells beginning around Stage 15 (Barish, 1986, 1991; O'Dowd, Ribera & Spitzer, 1988; Moody-Corbett et al., 1989; Moody-Corbett & Gilbert, 1990; Spruce & Moody, 1992), which we define as 0 hr. Although different cell types cannot be identified morphologically at earlier times, in control recordings we found no cells that exhibited delayed rectifier-type potassium currents, or any other voltage-dependent currents during a 12-hr period before Stage 15 (n = 25, Figs. 1*B* & 2*A*). Two types of control were used; uninjected embryos were used mainly but for some experiments embryos injected with a heterogenous RNA of unknown composition were also used. In cells isolated from the latter embryos, the profile of potassium current expression did not differ from uninjected embryos (*data not shown*).

Following injection of Kv1.1 cRNA into fertilized eggs, however, a delayed rectifier-type potassium current was seen in 42% (33/79) of gastrula/neurula cells during the same 12-hr period with an average density of 19.8 ± 3.7 pA/pF (n = 33) (Figs. 1*A* and 2*A*). Although no recordings were made earlier than 12 hr before Stage 15 (5.5 hr after fertilization), it is unlikely that the current which results from the expression of injected Kv1.1 cRNA ($I_{K,I}$) is present at these times since over the time interval of −12 to −10 hr only 1/7 cells expressed $I_{K,I}$ and the density in this cell was very small (0.5 pA/pF).

In control embryos, the endogenous delayed rectifier potassium current ($I_{K,X}$) begins to appear in muscle cells at Stage 15 + 2 hr (Figs. 1*D* and 2*A;* Spruce & Moody, 1992). This is earlier than observed by Ribera & Spitzer (1991) by about 6 hr but the reasons for this have been discussed previously (Spruce & Moody, 1992). To examine the expression of $I_{K,I}$ in muscle and also because our raw data suggested that Kv1.1 expression might alter the normal developmental pattern of $I_{K,X}$ expression, we wanted to separately identify $I_{K,I}$ and $I_{K,X}$ in cells from injected embryos. This was possible because $I_{K,I}$ expressed at very early developmental times activates at a more negative voltage than $I_{K,X}$ (Fig. 2*B*), though at a similar voltage to mKv1.1 current expressed in Chinese hamster ovary cells (Robertson & Owen, 1993). The difference in voltage dependence was quantified as follows. The ratio of the total outward potassium current at −28 mV to that at +22 mV was measured. For "pure" currents, the current ratios (R) were measured in control muscle cells (2 to 8 hr after Stage 15) for $I_{K,X}$ ($R_{K,X}$), and very early blastomeres (4 to 12 hr before Stage 15) for $I_{K,I}$ ($R_{K,I}$): $R_{K,X}$ = 0.02 ± 0.02 (±SD; n = 43); $R_{K,I}$ = 0.33 ± 0.08 (±SD; n = 13), the difference being highly significant ($P < 10^{-31}$; t-test). We used twice the standard deviation away from each mean to define the limits of the populations of current ratios for the "pure" currents. Therefore, the range of current ratio values lying in between the two populations (>0.06 and <0.17) would indicate ionic currents which are mixtures of $I_{K,I}$ and $I_{K,X}$. A problem with this analysis is that it subsequently

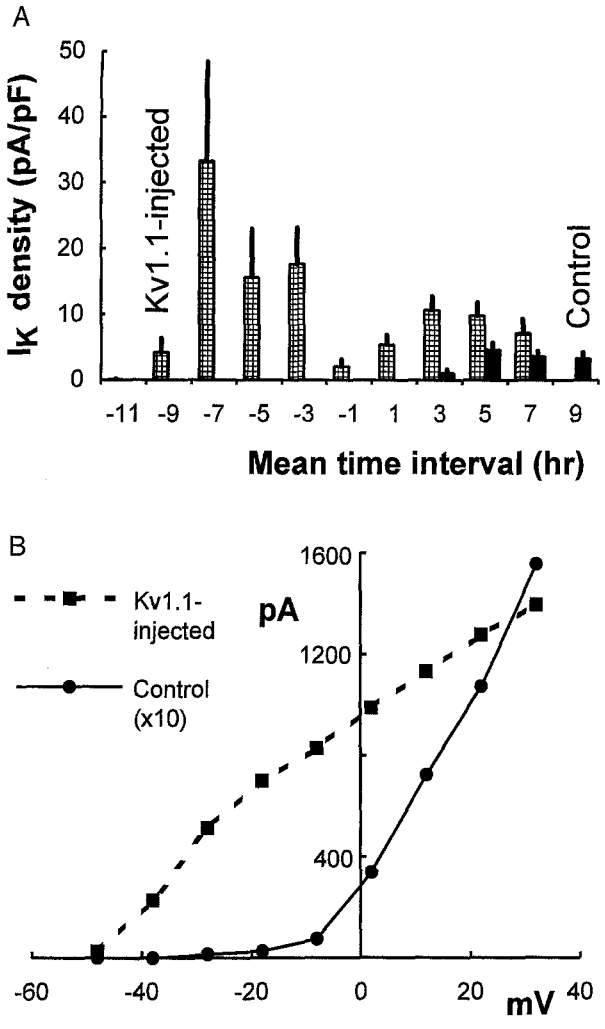

Fig. 2. Expression of delayed rectifier potassium current in injected and control embryos. (*A*) Mean outward potassium current densities over 2-hr time intervals (−12 to −10 hr, etc., relative to Stage 15) are plotted for all cells isolated from Kv1.1-injected embryos (hatched bars) and cells isolated from control embryos (solid bars). Standard error bars are shown. For each time interval the number of cells expressing current over the total number of recordings made are as follows: Kv1.1-injected—1/7 (−11 hr), 6/12 (−9 hr), 3/5 (−7 hr), 3/6 (−5 hr), 10/14 (−3 hr), 7/27 (−1 hr), 11/25 (1 hr), 17/23 (3 hr), 22/28 (5 hr), 9/13 (7 hr); Control—9/29 (3 hr), 16/20 (5 hr), 13/15 (7 hr), 4/5 (9 hr). No control cell expressed $I_{K,X}$ before 2 hr. (*B*) Difference in voltage-dependence of activation of $I_{K,I}$ and $I_{K,X}$. Current-voltage relations for the cells of Fig. 1*A* (Kv1.1-injected) and Fig. 1*D* (Control) are plotted in this figure. Current was measured at the end of the 800 msec voltage steps. To enable easier comparison, each control current value has been multiplied by 10.

shows that the expression of $I_{K,X}$ is accelerated in injected embryos so it cannot be known with certainty even at early developmental times that cells contain $I_{K,I}$ alone. However, the raw current ratio data demonstrates values within a relatively narrow range before −4 hr but widely varying values subsequently (*data not shown*). This is why we used recordings made before −4 hr to calculate the current ratio for "pure" $I_{K,I}$. For mixtures, the absolute levels of each current type are calculated by assuming that $R_{K,I}$ is equal to the mean, 0.33, and that $I_{K,X}$ contributes insignificantly to the total current at −28 mV ($I_{(-28\ mV)}$). So,

$$I_{K,I(+22\ mV)} = I_{(-28\ mV)}/0.33$$

$$I_{K,X(+22\ mV)} = I_{(+22\ mV)} - I_{K,I(+22\ mV)}$$

where $I_{(x\ mV)}$, $I_{K,I(x\ mV)}$ and $I_{K,X(x\ mV)}$ refer to the current densities of total current, Kv1.1 current and endogenous delayed potassium current, respectively, at the indicated voltages.

Effects of $I_{K,I}$ Expression on the Development of $I_{K,X}$

Figure 3 shows the results of such an analysis where $I_{K,I}$ and $I_{K,X}$ densities are plotted separately as functions of development in cells from embryos injected with Kv1.1 cRNA and compared to the expression of $I_{K,X}$ from control embryos. Thus, $I_{K,I}$ continues to be expressed in muscle cells from injected embryos after Stage 15, although at a density less than the peak achieved at early developmental times. Nevertheless, the same percentage of cells express $I_{K,I}$ at later times; 38% (18/48) at +2 to +8 hr compared with 38% (30/79) at −12 to 0 hr. This suggests that, since a heterogeneous population of cells is being examined at the early times and muscle cells only at the later times, there is probably no cell-specific localization of cRNA.

A surprising effect predicted by our analysis of current ratio data is that $I_{K,X}$ appears at much earlier developmental times; although, we cannot exclude the possibility that biochemical modification of Kv1.1 channels alters their voltage dependence (*see* Discussion). From −4 to +2 hr, 12/29 current-containing cells expressed $I_{K,X}$ at a mean density of 9.7 ± 2.1 pA/pF and, in fact, 7 cells appeared to contain $I_{K,X}$ alone, since the current ratios fell within the distribution for "pure" $I_{K,X}$. The large separation of the distributions of current ratios for $I_{K,I}$ and $I_{K,X}$ (*see above*) predicts that the chance that even only one of these cells contained "pure" $I_{K,I}$ is very small ($P < 0.001$). A second effect of Kv1.1 injection on the expression of endogenous potassium current that is derived from the above calculations is that, from +2 to +8 hr, the density of $I_{K,X}$ in muscle cells from injected embryos is twice that of control muscle cells; 10.0 ± 1.0 pA/pF (n = 43) and 4.7 ± 0.6 pA/pF (n = 38), respectively ($P < 0.0001$, t-test). The percentage of all muscle cells which contain $I_{K,X}$ is only marginally increased, however (58% for control and 67% for injected embryos).

Effect of Expression of $I_{K,I}$ on Electrical Development of Other Currents in Muscle

Expression of $I_{K,I}$ also had a slight, but significant and unambiguous, effect on the development of the inwardly

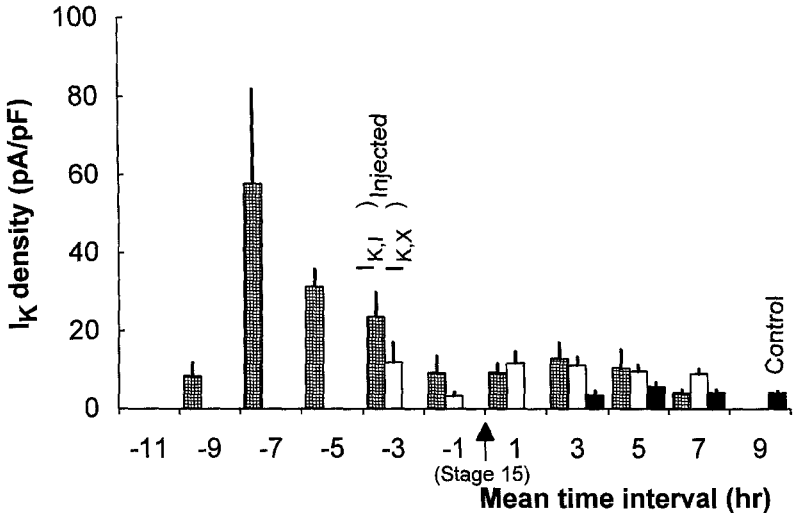

Fig. 3. Developmental expression of $I_{K,I}$ and $I_{K,X}$. Mean outward potassium current densities for current-containing cells from Kv1.1-injected and control embryos. For injected embryos, $I_{K,I}$ and $I_{K,X}$ were separately identified (hatched and open bars, respectively) and quantified using the procedure detailed in the Results. The solid bars plot the data for control embryos. For each average, n = 3–20.

rectifying potassium current (I_{IR}; Fig. 4). I_{IR} is normally the first current expressed during muscle cell development in *Xenopus,* beginning at around Stage 15 (Spruce & Moody, 1992). During the first 2 hr after Stage 15, cells from Kv1.1-injected embryos expressing $I_{K,I}$ were more likely to express detectable I_{IR} than cells with no $I_{K,I}$ (57% (4/7) *vs.* 16% (3/19)) but this difference was not significant ($P < 0.1$, Fisher's exact test (Fisher, 1958)). At slightly later times (2–4 hr), however (when the number of I_{IR}-expressing cells is greater), I_{IR} density was significantly increased in $I_{K,I}$-expressing cells: 2.9 ± 0.5 pA/pF ($n = 6$) *vs.* 1.4 ± 0.3 pA/pF ($n = 10$; $P < 0.01$). Figure 4 combines the effects of $I_{K,I}$ on both the proportion of cells which express I_{IR} and its density by averaging results from all cells over 4-hr time intervals. At the later time interval, $I_{K,I}$ expression had no effect on I_{IR} density.

Sodium current (I_{Na}) is expressed normally in only few isolated myocytes over the time period used in this study (Spruce & Moody, 1992). There was no effect of $I_{K,I}$ on this expression pattern (*data not shown*).

Comparison of Kv1.1 Current Properties with Other Expression Systems

In early blastomeres (before changes in current ratio), $I_{K,I}$ differs in two additional respects from Kv1.1 current seen after RNA injection into *Xenopus* oocytes (Hopkins et al., 1994) and after stable transfection of cloned mKv1.1 channels in Chinese hamster ovary (CHO) cells (Robertson & Owen, 1993). First, the current in blastomeres often had an inactivating component (Fig. 1*A* and *C*) whereas in the other cell types it does not. Although the fraction of $I_{K,I}$ which inactivates over 155 msec was variable (*cf.* Figs. 1*A* and 5*A*), on average, it was substantial (0.12 ± 0.02; $n = 35$; −12 to +8 hr) and there was no significant change during development

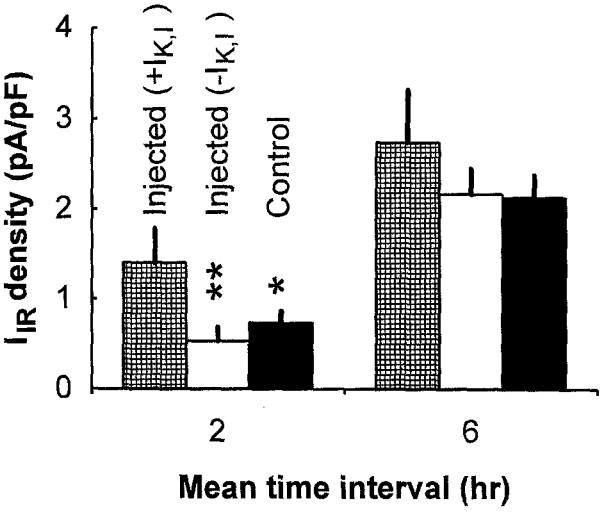

Fig. 4. $I_{K,I}$ modulates expression of I_{IR} in muscle cells. The average I_{IR} density over 4-hr time intervals (0 to 4 and 4 to 8 hr) is plotted for: cells from injected embryos which contain $I_{K,I}$ (hatched bars; mean times of recording = 2.0 ± 0.4 hr ($n = 15$) and 5.4 ± 0.3 hr ($n = 10$)); cells from injected embryos which do not contain $I_{K,I}$ (open bars; 1.6 ± 0.2 hr ($n = 34$) and 5.6 ± 0.1 hr ($n = 30$)); and cells from control embryos (solid bars; 2.5 ± 0.2 hr ($n = 38$) and 5.8 ± 0.2 hr ($n = 34$)). Standard error bars are shown. Asterisks indicate a significant reduction in the density of I_{IR} when compared to $I_{K,I}$-containing cells (*: $P < 0.03$; **: $P < 0.001$).

(*data not shown*). The endogenous potassium current inactivates as well (Fig. 1*D*), but to a greater extent (0.19 ± 0.03; $n = 37$; control embryos). For this analysis, only cells from injected embryos identified from current ratio data as containing "pure" $I_{K,I}$ were used. Thus, early expression of $I_{K,X}$ probably does not explain this discrepancy in current properties between embryos and other expression systems.

A second difference we observed between Kv1.1 currents expressed in blastomeres *vs.* oocytes and cell lines is in their sensitivity to the I fraction of Dendro-

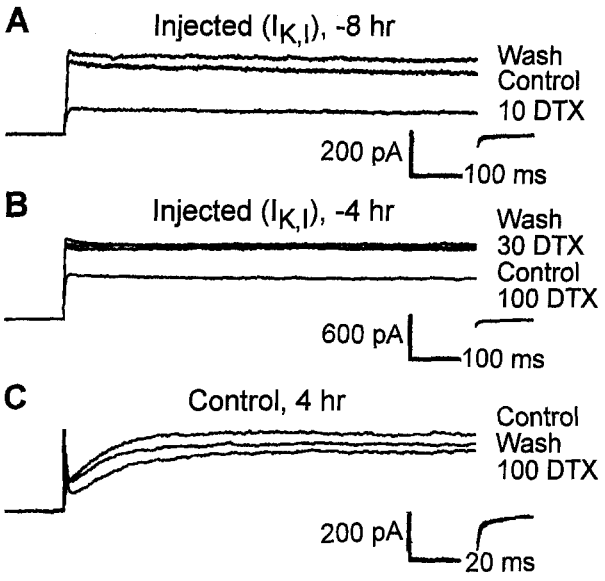

Fig. 5. Block of potassium current by Dendrotoxin-I. Each of the three sets of current traces are recordings from different cells. For each set of traces, the cell was held at −98 mV and step depolarizations to +32 mV were applied before (control), during application of the indicated amount of DTX-1 to the cell either from a spritzing electrode or by exchanging the bath solution, and after (wash) DTX-I had been removed by exchanging solutions in the bath. (*A*) Cell from Kv1.1-injected embryo 8 hr before Stage 15 identified by current ratio measurement as containing "pure" $I_{K,I}$. (*B*) Cell from Kv1.1-injected embryo 4 hr before Stage 15 identified as containing "pure" $I_{K,I}$. (*C*) Cell from control embryo 4 hr after Stage 15 (note the different time scale for the current traces compared to *A* and *B*).

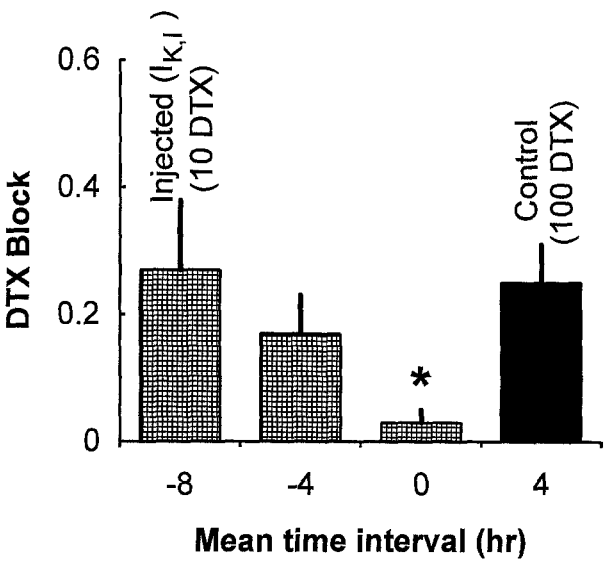

Fig. 6. Sensitivity of block of potassium current by DTX-I is related to developmental time. Fractional block of potassium current at +32 mV by the indicated concentration of DTX-I is averaged over 4-hr time intervals (−10 to −6 hr, etc., relative to Stage 15) for cells expressing "pure" $I_{K,I}$ from Kv1.1-injected embryos (hatched bars) or from control embryos at times when $I_{K,X}$ is expressed (solid bar). Standard error bars are shown. Asterisk indicates a statistically significant decline in the blocking effectiveness of DTX-I ($P < 0.02$). For each average, n = 5–10.

toxin (DTX-I). Kv1.1 currents are blocked very efficiently by DTX-I in *Xenopus* oocytes (K_i = 3 nM; Hopkins et al., 1994) and CHO cells (Robertson & Owen, 1993). In blastomeres, however, the effect of DTX-I on currents identified as "pure" $I_{K,I}$ was highly variable, even at the earliest stages examined and indeed, on average, it was much less effective (Figs. 5*A, B* and 6). Thus, from −10 to −6 hr, the fraction of $I_{K,I}$ blocked by 10 nM DTX-I was 0.27 ± 0.11 (n = 7), although in two cells the current block was similar to that in oocytes (for example, *see* Fig. 5*A*). Despite this variability at the early stages, there is a statistically significant decline in the sensitivity of the current as development progresses (Fig. 6). The endogenous delayed potassium current in *Xenopus* muscle is relatively insensitive to DTX-I (Figs. 5*C* and 6): DTX-I concentrations as high as 100 nM achieve only about a 20% block. The early expression of $I_{K,X}$ cannot be implicated in the declining sensitivity of $I_{K,I}$, however, since those cells tested contained only "pure" $I_{K,I}$. The sensitivity of $I_{K,X}$ expressed at early times to DTX-I has not been tested.

Discussion

Following injection of Kv1.1 cRNA into fertilized *Xenopus* eggs (one-cell stage), we examined the expression of potassium currents in unidentified blastomeres during a 12-hr period prior to Stage 15 and also in developing muscle cells (but not other cells types) for at least 7 hr subsequently. Our data show that, before Stage 15, a high proportion of gastrula and early neurula cells express a delayed rectifier-type potassium current ($I_{K,I}$) when control cells normally express no voltage-dependent currents. In muscle cells after Stage 15, the exogenous current continues to be expressed, although at lower densities. There are two questions which arise from this kind of study. First, does embryonic misexpression create a current with unchanged characteristics compared to other expression systems? Second, does embryonic misexpression alter normal development?

At times when control blastomeres do not express voltage-dependent currents, $I_{K,I}$ from injected embryos was markedly different in terms of inactivation, DTX-sensitivity and voltage dependence from Kv1.1 current expressed in *Xenopus* oocytes (Hopkins et al., 1994) and CHO cells (Robertson & Owen, 1993). These modified characteristics were not consistently seen, however, and could not be explained by a common mechanism since there was no correlation between them. Despite this, altered biochemical processing of the Kv1.1 protein could explain some of the differences between embryonic cells and oocytes or mammalian cell lines. Effects on current kinetics have been observed for other potassium channels when embryonic and oocyte expression systems were compared (Zagotta, Hoshi & Aldrich, 1989) and

"shifts" in the voltage dependence of gating of calcium channels by second messenger modulation have been described (Bean, 1989; Beech, Bernheim & Hille, 1992). In fact, if the voltage dependence of Kv1.1 current were altered then our analysis of endogenous delayed potassium current ($I_{K,X}$) expression would be incorrect (*see below*). However, this interpretation would have to explain both the presence in early blastomeres of Kv1.1 current whose voltage dependence and kinetics were altered so much that it was indistinguishable from $I_{K,X}$ and, conversely, Kv1.1 current with unaltered gating at later times in muscle cells. These data are inconsistent with the appearance and maturation of a biochemical process. Finally, even within a single expression system, variation in channel density can affect toxin sensitivity (Moran et al., 1992; Honore et al., 1992). Our data do not support this association, though, since the density of outward potassium current is not correlated with any of the altered properties we describe.

The second issue is whether endogenous electrical development is modulated by Kv1.1 expression. This is indeed so. Inward rectifier potassium current (I_{IR}) is significantly upregulated in muscle cells when $I_{K,I}$ is present. Indirect support is also provided from our interpretation of the altered voltage dependence of $I_{K,I}$. The exogenous current can be distinguished from $I_{K,X}$, which appears in control muscle cells starting about 2 hr after Stage 15, by activation at much more negative potentials. Based on this difference in voltage dependence, $I_{K,I}$ was separated from $I_{K,X}$ revealing that, in Kv1.1-injected embryos, $I_{K,X}$ appeared up to 6 hr earlier than normal and, at later stages in identified muscle cells, was present at higher density than in control embryos. We should emphasize that, prior to Stage 15, it is possible that the cells from injected embryos which contain "$I_{K,X}$" are not muscle precursors. Clearly, this analysis assumes that the embryonic cell does not modify the voltage-dependent gating of Kv1.1 channels. Nevertheless, the characteristics of the current in early blastomeres where $I_{K,I}$ is absent are not distinguishable from those of $I_{K,X}$ from control muscle cells.

How might endogenous electrical development have been modulated by Kv1.1 expression? Perhaps functional Kv1.1 channels are required in the plasma membrane at early stages, such that their presence affects the patterns of spontaneous depolarizations that would otherwise occur during early development. In other experiments, we have shown that creating aberrant patterns of electrical activity during early development, by overexpression of a cloned Na channel, can indeed affect $I_{K,X}$ development (Linsdell & Moody, 1994). We have no independent evidence, however, for the normal existence of such activity in the early embryo. It is also possible that the effects of Kv1.1 expression occur during processing of the channel proteins prior to insertion in the plasma membrane. Thus, $I_{K,X}$ and I_{IR} channel proteins or RNA might be present much earlier in development than the functional channels appear (e.g., Ribera & Spitzer, 1989) but are triggered to be expressed early by the processing of Kv1.1 cRNA. Hybrid (mouse/*Xenopus*) delayed potassium channels could even be generated initially. The variable expression of an inactivating component could result from association of Kv1.1 channels with an accessory *Xenopus* potassium channel subunit (Rettig et al., 1994). Altered DTX-sensitivity and voltage dependence are difficult to explain by heteromultimer formation, however. MacKinnon (1991) (*see also,* Hopkins et al., 1994) predicts that heteromultimers of 1 toxin-sensitive and 3 toxin-insensitive subunits would have a 4× lower toxin sensitivity. Further, channels formed from subunits with additional differences in their intrinsic voltage dependence should have predictable alterations in their gating (Tytgat & Hess, 1992). But, our data demonstrate at least a 20× variation in the sensitivity of potassium current to DTX-I and we do not find changes in current ratio for currents whose DTX-I sensitivity is very low. Although $I_{K,X}$ and Kv1.1 currents activate at very different rates (Ribera & Spitzer, 1991; Robertson & Owen, 1993), analysis of current kinetics could not examine the possibility of heteromultimeric channels because of the variable expression of the fast activating, inactivating current component (Fig. 1; *see also,* Spruce & Moody, 1992; Moody-Corbett & Gilbert, 1992). Although, we have much stronger evidence for the modulation of I_{IR} expression, major structural differences would preclude subunit interactions.

Whatever the causes of the substantial modulation of currents we describe, it is perhaps not surprising that an embryonic cell which has to coordinate considerable changes in the functional expression of its own ion channels would be markedly influential over that of exogenous ion channels. Hence, when the *Xenopus* embryo is employed purely as a heterologous expression system to study properties of an exogenous channel, the results should be interpreted cautiously (Honore et al., 1994). The embryo does indeed have several distinct advantages over the oocyte as an expression system for ion channels: (i) high density currents are expressed rapidly, within 7 hr after injection, probably reflecting much higher rates of protein synthesis after fertilization; (ii) a single injection yields a large number of cells that are easily dispersed (the *Xenopus* gastrula contains more than 20,000 cells); and (iii) dispersed gastrula cells are clean and relatively small, and therefore are easily voltage-clamped and dialyzed. Taken together, however, our data suggest that embryonic expression may be more appropriate as a tool to study ion channel development than as a passive expression system.

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