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A STUDY OF THE DEVELOPMENTAL CHANGES IN OUTWARD CURRENTS OF RAT VENTRICULAR MYOCYTES

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SUMMARY

1. In ventricular muscle of rat heart, the action potential undergoes a major developmental change in shape in the days and weeks immediately after birth. Potassium (K^+) currents which may affect the shape of the action potential have been studied using a whole-cell voltage-clamp technique with single cells from the ventricles of rats aged 1–10 days. All recordings were made at 22–23 °C.

2. Three discrete ages were chosen, 1-day (cells isolated within 24 h of birth), 5-day and 10-day rats. These parallel the developmental action potential shortening from neonatal towards adult type. Action potentials of single myocytes were initially of long duration at 1 day with a prominent plateau phase, but had shortened somewhat by 10 days of age. The 5-day group exhibited an action potential transitional in character between the earlier and later groups of cells.

3. Potassium current blocking agents were used to assess the importance of the various outward K^+ currents for the action potential waveform at different ages. 4-Aminopyridine (4-AP; 2×10^{-3} M) which preferentially blocks voltage-activated transient outward currents affected action potentials at all ages, but increases in duration were most pronounced in the 10-day group. Only a small prolongation of the initial phase of repolarization of 1-day action potentials was seen. Extracellular barium chloride, $0{\cdot}1$–2×10^{-3} M, a blocker of inwardly rectifying potassium channels, had a marked slowing effect on repolarization in all the three age groups. Resting membrane depolarization was also produced by barium.

4. Developmental changes in the inwardly rectifying background current (I_{K1}) and the cardiac transient outward current, I_t, were investigated. I_{K1} was recorded as the current sensitive to 2×10^{-3} M-$BaCl_2$ during voltage-clamp steps from a holding potential of -90 mV. It was found to decrease in magnitude, approximately by a factor of three, from 15 to 5 pA/pF during the first ten postnatal days. This reduction can explain the maturational slowing of repolarization during the final phase of the action potential in rat heart.

5. Current–voltage relations for I_{K1} from the three age groups crossed at the zero current potential at ~ -90 mV, near the calculated V_K for the pipette filling solution

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and an external bath K^+ concentration of 5×10^{-3} M. This suggests that I_{K1} channels in these cells are quite selective for K^+ ions and that developmental changes in the potassium selectivity are not responsible for changes in I_{K1}.

6. Voltage-clamp steps from less negative holding potentials revealed that this maturational change in I_{K1} was mediated (in part) by changes in the kinetic properties of I_{K1}. The voltage-dependence of inactivation of I_{K1} was shifted to less negative potentials during development. Boltzmann functions fit to data showed slope factors between +22·7 mV at 1 day and +18·4 mV at 10 days. However, $V_{\frac{1}{2}}$ the potential at which half-inactivation occurs was −136 mV in 1-day cells and shifted to −95·6 mV in 10-day cells. At more negative potentials than −100 mV the inactivation of I_{K1} was best fit by a dual exponential process, consisting of fast and slow components. Neither component was strongly voltage-dependent, nor age-dependent. There were significant differences in the amplitudes of fast (A_2) and slow (A_1) components of inactivation. With development, the ratio $A_2/(A_1+A_2)$ increased from 0·48 to 0·67, $P < 0{\cdot}001$.

7. Transient outward current, I_t, was recorded at potentials positive to −30 mV and showed significant developmental changes across the age groups studied. A four-fold increase in I_t was observed from 1- to 10-day cells. This increase appeared solely due to increase in the magnitude of a voltage activated transient outward current as the effects were not changed by abolition of the Na^+ current by tetrodotoxin (TTX, 3×10^{-5} M) or abolition of any putative Ca^{2+}-activated transient outward current using $CdCl_2$ (3×10^{-4} M).

8. The increase in transient outward current could not be attributed to changes in the voltage-dependence of steady-state inactivation. This was fitted well to a Boltzmann distribution where $V_{\frac{1}{2}}$ was −59·9 mV with a slope factor of −15·1 mV. The low current density of I_t at birth may be attributed in part to an initial poor selectivity of I_t for K^+ ions. Mean V_{rev} at 1-day was −28·3 mV and increased to −53·4 mV at 10 days.

9. We hypothesize that developmental changes in outward membrane currents play an important role in the changes of neonatal rat ventricular cell action potentials with age, notwithstanding any changes in inward currents that may occur. Increases in I_t, which may be attributable to changes in the ionic selectivity of the channel occur in parallel with the gradual acquisition of the rapid initial phase of repolarization characteristic of the action potential of mature rat ventricle. Changes in the inward rectifier, I_{K1}, may be responsible for changes in the final phase of repolarization of rat ventricular cell action potentials and in the genesis of the resting potential.

INTRODUCTION

One of the remarkable features of the cardiac action potentials is its long duration which can be two orders of magnitude longer than nerve action potentials. Since the mammalian cardiac action potential plateau represents the delicate balance of a number of depolarizing and repolarizing ionic currents it is difficult to decide which particular ionic currents are predominantly responsible for controlling action potential repolarization. The rat ventricular action potential is unique among the ventricular tissues of mammals in having an action potential that is less than 100 ms

long (Weidmann, 1956). There is almost no recognisable plateau phase except one that appears at very negative potentials and which has been attributed to changes in electrogenic Na^+–Ca^{2+} exchange current (Schouten & ter Keurs, 1985). This tissue provides a suitable model for assessing the importance of ionic currents underlying repolarization, as ventricular muscle cells from prenatal and newborn rats have an action potential with an elevated plateau phase similar to that of other mammalian species (Couch, West & Hoff, 1969; Bernard, 1975). The postnatal shortening of the long neonatal rat action potential occurs over a time period of three weeks before the adult form is reached (Langer, Brady, Tan & Serena, 1975; Pučelík, Ježek & Barták, 1982).

In an attempt to understand this shortening, some authors have studied developmental changes in the inward currents of rat ventricle at the multicellular level (Van Ginneken & Jongsma, 1983) and using single myocytes (Brown, Fedida & Kilborn, 1986; Cohen & Lederer, 1988). These authors have concluded that a change in the voltage-dependence of inactivation of calcium current occurred during development that resulted in a calcium window current, present at birth, disappearing during development. This window current was present at plateau potentials and was thought to underlie the long action potential of the ventricular myocytes of newborn rats. Relatively little attention has been given to the outward membrane currents of rat heart cells and the role that changes in these currents might contribute to changes in action potential shape. During prenatal ontogenesis, there is indirect evidence for a growing role for K^+ currents in that action potentials from older cells are prolonged by tetraethylammonium ions (TEA) (Bernard, 1975). However, postnatally, TEA (2×10^{-2} M) causes a similar prolongation of action potentials in various age groups from neonatal to adult (Pučelík, Králíček, Barták & Ježek, 1983). If there are postnatal increases in outward current, they may overlap the inward depolarizing Ca^{2+} current and thus cause suppression of the plateau phase and shorten the action potential.

The aim of the present study was to characterize the developmental changes in the transient outward currents of rat ventricular myocytes. These currents are known to play an important role in modulating the repolarization phase of the action potential of a number of species (Giles & Imaizumi, 1988). In addition we have studied changes in the background potassium currents present in neonatal rat ventricular cells. We have found that changes in potassium current with development do occur. There is an increase in the transient outward current with age and there is a shift in the voltage-dependence of inactivation of I_{K1} that has the effect of reducing I_{K1} at diastolic potentials in older rats. Parallel changes in the action potentials of neonatal rat ventricular myocytes reflect these underlying changes in membrane current. Preliminary reports of some aspects of this work have appeared previously (Brown *et al.* 1986; Kilborn, Fedida & Brown, 1988).

METHODS

Cell isolation

Single cardiac myocytes were isolated from neonatal rats (Sprague–Dawley) in three specific age groups. These were 1 day, 5 days and 10 days of age; all rats used were within 24 h of these dates. Animals were killed by decapitation and hearts excised under sterile conditions into a filter

sterilized (0·2 μm) Tyrode solution containing, in mM: NaCl, 121; KCl, 5·0; sodium acetate, 2·8; $MgCl_2$, 1·0; glucose, 5·49; $NaHCO_3$, 24·0; Na_2HPO_4, 1·0; $CaCl_2$, 0·005; pH 7·4 at room temperature. After washing, the ventricles were minced in this solution containing 0·16 mg/ml collagenase (Yakult, Tokyo) for 1-day cells (0·23 mg/ml for 5-day and 0·33 mg/ml for 10-day) and 10 mg/ml albumin (Sigma A-2153) plus taurine, 2×10^{-2} M. Cells were incubated in a vial in a dry bath heater (Fisher) and agitated continuously at 37 °C. After a 10 min period the initial solution was discarded. Subsequently the tissue was incubated for five minute periods in 2 ml enzyme solution. After each period the supernatant was removed and the enzyme solution renewed. The supernatant samples were diluted 1:1 in a culture medium (see below) and centrifuged at 2000 r.p.m. for 3–4 min. The cells were resuspended and cultured in Ham's F-10 medium (Sigma, Type 1387) plus $NaHCO_3$, $1{\cdot}43 \times 10^{-2}$ M, 10% calf serum (Gibco), penicillin G (sodium) 100 units/ml and streptomycin sulphate 100 μg/ml, pH 7·35 with NaOH. Cells were plated onto glass cover-slips in culture dishes and maintained at 37 °C in a moist air CO_2 incubator until use.

In our early experiments (Brown *et al.* 1986) cells were isolated using a trypsin-based method (Mark & Strasser, 1966) in which trypsin (Type III, Sigma), 1 mg/ml, was dissolved in calcium-free Ham F-10 nutrient mixture (Gibco). However, there are reports that trypsin separation of single myocytes may cause ultrastructural damage to cells (Masson-Pévet, Jongsma & de Bruijne, 1976) and indeed we found that the collagenase method gave a higher and more consistent yield of healthy cells. Adult ventricular myocytes were prepared according to methods described by Piper, Probst, Schwartz, Hutter & Spieckermann (1982). These cells were used for electrophysiological study within 24 h of isolation.

Experimental procedures

After removal from the incubator, cover-slips with attached cells were transferred to culture dishes containing the modified Tyrode solution described above. Small portions of the cover-slip could then be transferred to the experimental bath where the adherent cells were superfused at 1 ml/min. When required, 4-aminopyridine (4-AP; Sigma) was used to block the voltage-activated transient outward current, I_t. Tetrodotoxin (TTX, Sigma) was used to block the inward Na^+ current, I_{Na}; all drugs were added to the superfusate at appropriate concentration. $CdCl_2$ was used to block Ca^{2+} channel currents and $BaCl_2$ to block inward rectifier current, I_{K1}. Neither $BaCl_2$ nor 4-AP were entirely specific for the K^+ currents that we used them to block. 4-AP (2×10^{-3} M), a concentration required to completely block I_t, caused a small reduction of I_{K1} under voltage clamp (data not shown) although this was not enough to affect the cells' resting potentials (cf. Fig. 2). $BaCl_2$ (2×10^{-3} M) was used in voltage-clamp experiments to ensure a rapid and complete block of I_{K1} during hyperpolarizing voltage-clamp steps, but lower doses were effective on steady-state currents (DiFrancesco, Ferroni & Visentin, 1984; Hirano & Hiraoka, 1988) and at concentrations less than 5×10^{-4} M had no effect on I_t (Josephson, Sanchez-Chapula & Brown, 1984; Giles & Imaizumi, 1988). In such experiments, HEPES-buffered bath solutions were used containing, in mM: NaCl, 121; KCl, 5·0; sodium acetate, 2·8; $MgCl_2$, 1·0; glucose, 10·0; HEPES, 10·0; $CaCl_2$, 1·8; pH 7·4 with 1 M-NaOH.

Whole-cell patch-clamp electrodes were drawn on a horizontal pipette puller (Soutter Instruments, California). In some experiments these were fire-polished before use to increase membrane seal resistance. Electrodes were of 2–5 MΩ resistance when filled with the following solution, in mM: potassium aspartate, 120; KCl, 30·0; Na_2ATP, 4·0; $MgCl_2$, 1·0; HEPES, 5·0; pH 7·2 with 1 M-KOH. Voltage-clamp experiments were performed using an Axopatch 1A patch clamp (Axon Instr.) Command signals were either generated from the amplifier or via a pulse generator (Galveston Electr. Corp). Measurements of electrode junction potentials were carried out as described previously (Fenwick, Marty & Neher, 1982). Mean corrections amounted to 10·8 mV for pipette solutions used in this study (pipette solution negative). As a first approximation, therefore, all data have been corrected by −10 mV. Capacitance of cells was measured by analogue compensation of the transient in response to 10 mV hyperpolarizing voltage-clamp pulses. Mean (±S.D.) cell capacitance was 9·4±2·4 pF ($n = 38$) at 1 day, 14·8±4·6 pF ($n = 45$) at 5 day and 16·5±6·5 pF ($n = 42$) at 10 days of age. When statistical comparisons are made between cells of different ages data have been normalized to cell capacitance.

All experiments were conducted at room temperature (22–23 °C). Current records were filtered at 5 kHz using a 4-pole Bessel filter and digitized at 1–10 kHz for on-line storage on a laboratory microcomputer. Subsequent analysis was carried out using dedicated programs on a D.E.C. Vax minicomputer or using commercially available PC-based spreadsheet and graphics software.

RESULTS

Action potentials

Typical action potentials recorded from 1-, 5- and 10-day rat cells are shown in Fig. 1. These were stimulated using 2 ms suprathreshold current pulses in current-clamp mode, not shown. The 1-day action potential is long with a prominent overshoot but only a small phase of rapid repolarization. The plateau is around 0 mV and there is a definite final phase of repolarization. At 5 days the action potential has shortened but still has a definite plateau region. By 10 days of age the action potential has a prominent initial phase of repolarization and a short plateau. There is a noticeable slowing of the final phase of repolarization in the 10-day cells and the action potential shape is becoming similar to the adult action potential which is extremely short at this stimulus frequency (Fig. 1*D*). These action potentials are consistent with measurements from intact preparations recorded previously (Langer *et al.* 1975; Powell, Terrar & Twist, 1980; Pučelík *et al.* 1982).

To assess the effects of repolarizing currents on the action potential of rat neonatal ventricular myocytes we have used 4-AP to block the voltage-activated transient outward current and $BaCl_2$ to block I_{K1}, background potassium conductance (see Methods). The changes in action potential shape induced by these agents are illustrated in Fig. 2. The control action potentials are typical for the age groups illustrated and characteristically the younger cells exhibit an action potential with a prominent plateau phase, whereas the 10-day and adult rat cells have short action potentials with almost no plateau phase. 4-AP prolongs all action potentials, with the prolongation in the adult rat ventricular action potential being most prominent. The effect on 1-day cells was restricted to prolongation of the initial phase of repolarization and little overall change in the action potential duration or cell resting potential was seen (Fig. 2*A*). In 5- and 10-day cells an intermediate situation is observed; a marked slowing of the initial phase of repolarization is accompanied by action potential prolongation when I_t is blocked. Note, however, that the effects on the action potential of 5- and 10-day neonatal cells are still much less than the effect on adult cells (Fig. 2*D*).

Exposure to 10^{-4} M-$BaCl_2$ causes a significant action potential prolongation in all age groups of cells (Fig. 2*A* and *C*). These changes were readily reversible on wash off of the $BaCl_2$ and indicate that background K^+ currents have a significant role to play in the repolarization of the action potentials of rat ventricular cells of all ages. The effects of $BaCl_2$ on the action potential are most important at potentials negative to -30 mV and indicate that the presence of I_{K1} is essential during the final phases of repolarization and for the maintenance of the resting potential in these cells. In six 1-day cells $0{\cdot}5$–2×10^{-3} M-$BaCl_2$ caused a 28·6 mV mean depolarization ($\pm 6{\cdot}8$ mV, S.D.) of the resting potential compared with $19{\cdot}7 \pm 7{\cdot}1$ mV in 10-day cells and $20{\cdot}8 \pm 7{\cdot}1$ mV in adult cells. The differences between these means were not significant at the 5% level.

Background potassium currents

In view of the effects of $BaCl_2$ on the action potentials of 1-, 5- and 10-day cells the inwardly rectifying background potassium current, I_{K1}, was studied during development. A typical example of recorded currents from a 1-day cell are shown in

Fig. 3*A* and *B*. The original records in Fig. 3*A* illustrate important features of I_{K1} in these cells. Under control conditions, hyperpolarizing clamp steps elicit large inward currents that show partial inactivation during the voltage pulse. Exposure to Ba^{2+} ions (Fig. 3*B*) virtually abolishes all current at negative potentials. At more positive

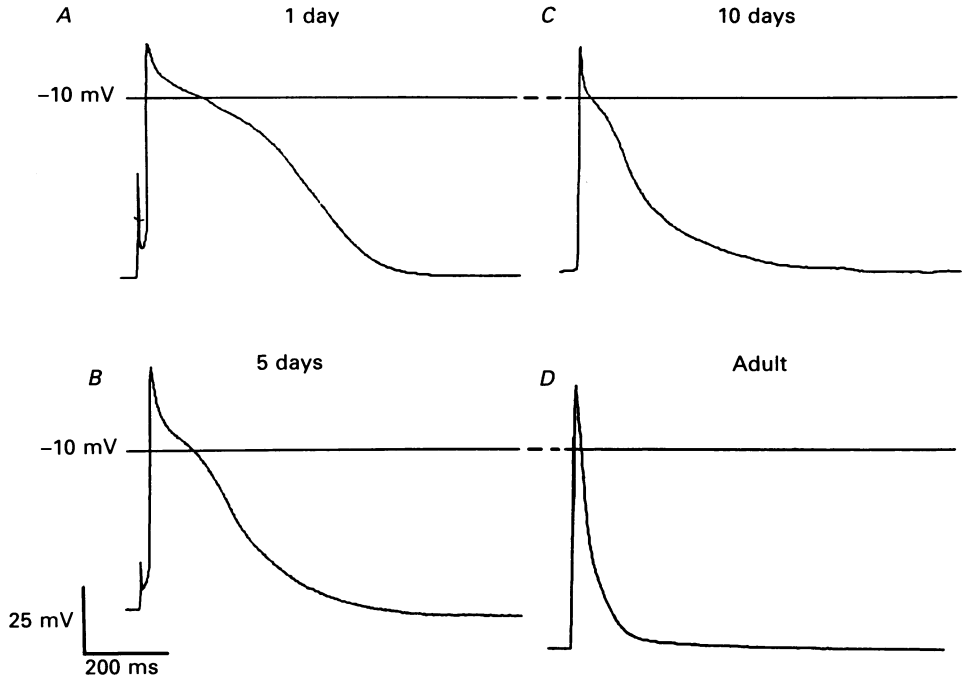

Fig. 1. Representative examples of action potentials recorded from rat ventricular myocytes of differing ages. Action potentials were elicited using 2 ms suprathreshold current pulses given at 0·2 Hz. The horizontal line depicts −10 mV in each case. *A*, 1 day; *B*, 5 days; *C*, 10 days; *D*, adult.

potentials currents are smaller and relatively unaffected by $BaCl_2$ (Fig. 3*B*, right panel) which suggests that little background current is present.

The graph in Fig. 3*C* summarizes these data. Instantaneous current–voltage relations are shown in the left graph. The barium-sensitive current shows significant inward current at negative potentials and marked inward rectification at potentials more positive than −80 mV. In this 1-day cell there is no background current at positive potentials and a region of negative slope conductance between −60 and −10 mV. The current remaining during Ba^{2+} exposure (■) is fairly linear, reversing at −30 mV and increasing somewhat in slope at positive potentials. This slight increase at positive potentials may represent a slowly inactivating portion of transient outward current at the end of the 400 ms voltage steps used to measure background current. The steady-state currents at the end of voltage-clamp pulses are plotted in the graph in Fig. 3*D*. Inactivation of background current was observed at negative potentials (Fig. 3*A*). I_{K1} is reduced by 10–20% during hyperpolarizing

voltage-clamp pulses (cf. Fig. 5). This inactivation becomes increasingly important as the pulse potential is made more negative and eventually, at −150 mV, less current is present in the steady state than at −120 mV.

These characteristics, barium-sensitivity, inward rectification, inactivation of inward currents and negative slope conductance suggest that the Ba^{2+}-sensitive

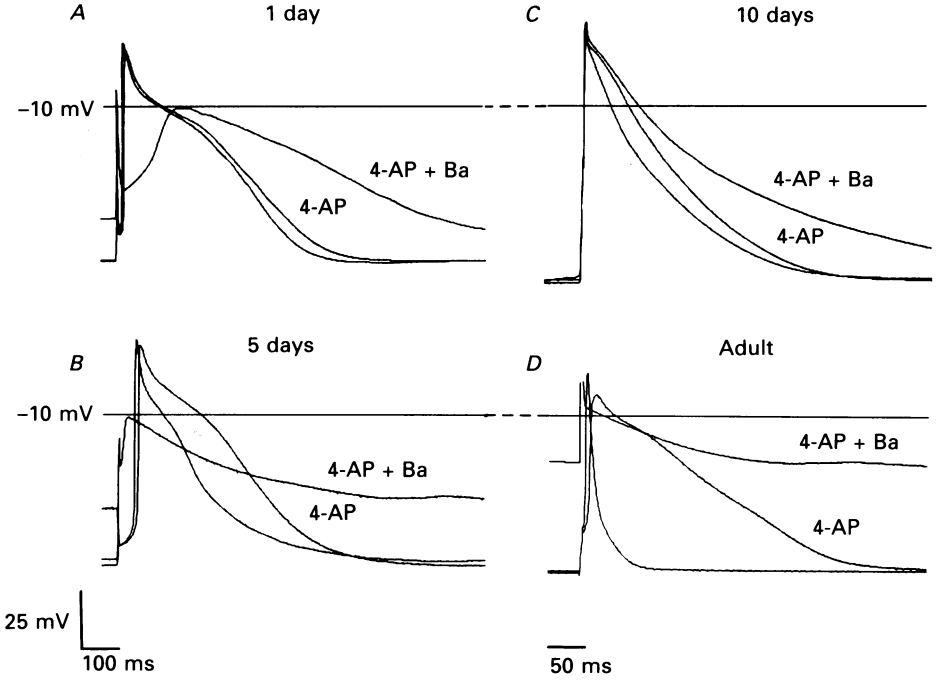

Fig. 2. The effects of 4-AP and $BaCl_2$ on the rat ventricular action potential. *A–D*, action potentials recorded from single rat ventricular cells of different ages as indicated. Action potentials were generated using 2 ms suprathreshold current pulses. Cells were stimulated at 0·1 Hz. Control action potentials were recorded (unlabelled traces) and cells exposed to 2×10^{-3} M-4-AP and then 2×10^{-3} M-$BaCl_2$ (except *A*, where $BaCl_2$ concentration was 10^{-4} M). The horizontal line in each panel represents −10 mV. Note that the action potentials in *C* and *D* are presented on a 2× faster time base than those in *A* and *B* as indicated by the time marks under each panel.

currents shown in Fig. 3 reflect K^+ ion movement through I_{K1} channels. The age characteristic of these currents are shown in Fig. 4. The peak Ba^{2+}-sensitive currents recorded during clamp pulses, are shown in Fig. 4*A*. The most striking feature of these data is the clear age-dependent *decrease* in instantaneous current, especially apparent at potentials negative to −40 mV. Data from all three ages show marked inward rectification, very little outward current at positive potentials and at least in the case of 1- and 5-day cells, a region of negative slope conductance is clearly apparent. In all cells studied in this way, the holding potential from which clamp pulses were given was −90 mV. Thus at −80 mV, near the cells' resting potentials (Figs 1 and 2) there is significant outward current only in the 1-day cells. The relations obtained from all three age groups cross at −90 mV which is very near the

zero current potential in each case and near V_K calculated for the internal and external solutions (−85 mV). The scaling of the three relations in Fig. 4 is approximately 3:2:1 from 1-, 5- and 10-day cells when data are normalized to cell capacitance.

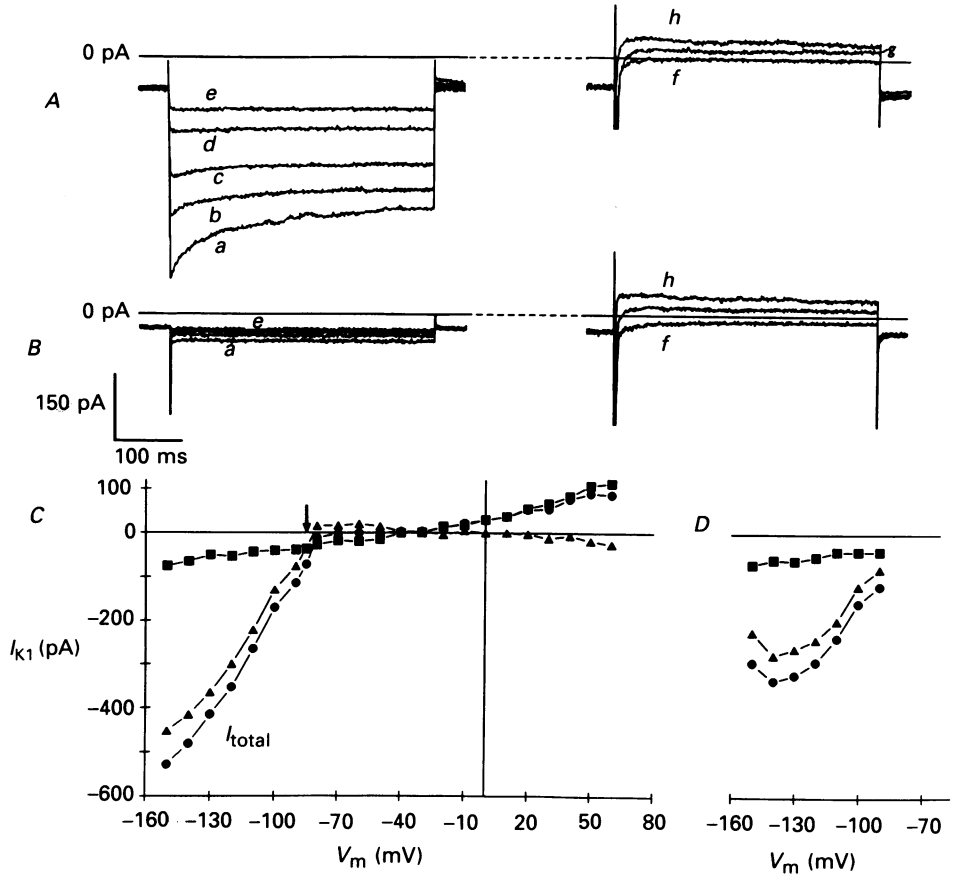

Fig. 3. Background K^+ current in 1-day rat neonatal cells. *A*, control membrane currents recorded during 400 ms voltage-clamp steps to (mV): *a*, −140; *b*, −120; *c*, −110; *d*, −100; *e*, −95; *f*, −50; *g*, −10; *h*, +10. *B*, current traces during exposure to 2×10^{-3} M-$BaCl_2$, same voltage steps as in *A*. All data obtained at a constant clamp pulse frequency of 0·1 Hz from a holding potential of −90 mV. The horizontal lines in *A* and *B* depict 0 pA. *I–V* diagrams of the data in *A* and *B* are shown in *C* for the instantaneous current and *D* for the steady-state current levels. In *C* the peak instantaneous current (cf. *A*) is shown as I_{total} (●). The barium-*resistant* current (cf. B) is shown as ■. The difference current, I_{K1}, is Ba^{2+}-sensitive (▲). In *D* the end-of-pulse currents for clamp pulse potentials between −150 and −100 mV are shown. The symbols for the data are as in *C*. The arrow in *C* denotes predicted V_K from internal and external K^+ concentration.

Quite a different relation is obtained when the steady-state I_{K1} data at different ages are plotted as a function of pulse potential (Fig. 4*B*). From −100 to −150 mV where significant inactivation during the hyperpolarizing voltage-clamp steps was seen to occur (cf. Fig. 3), the 10- and 5-day relationships have a shallow voltage-

dependence, and an increase in steady-state I_{K1} is only seen in 1-day cells. This last group showed less inactivation during hyperpolarizing clamp steps although some variability between cells was apparent as evidenced by the large error bars on 1-day points in Fig. 4*B*. This point is dealt with in more detail in subsequent figures.

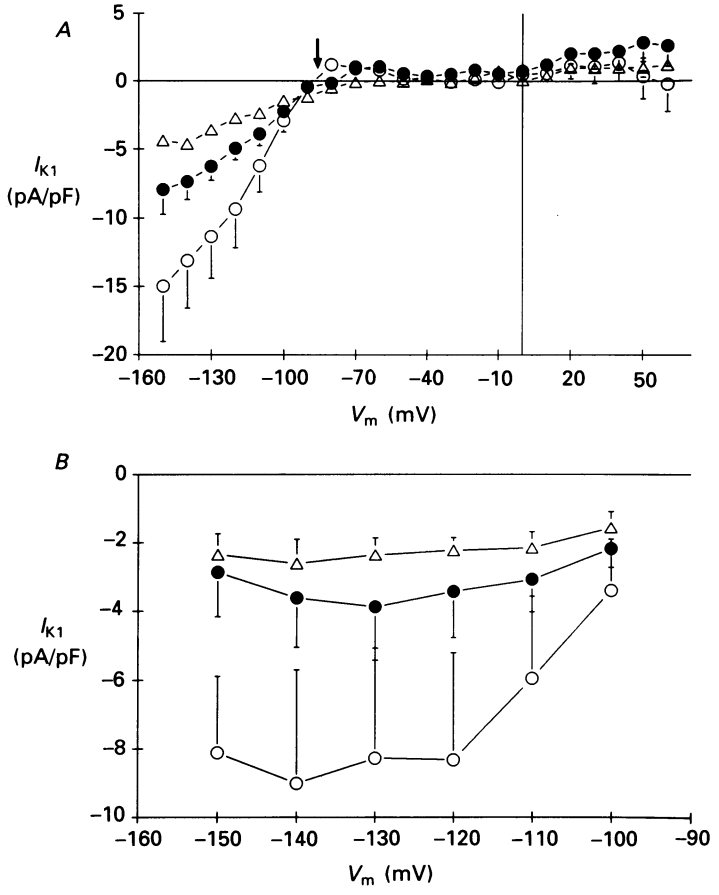

Fig. 4. Normalized current–voltage diagrams for I_{K1} from neonatal rat ventricular cells. Accumulated data for $BaCl_2$-sensitive currents (I_{K1}) from 1-, 5- and 10-day cells. Data are expressed as pA/pF, normalized to cell capacitance (see Methods). All data collected from a holding potential of −90 mV during 400 ms voltage-clamp steps given at a frequency of 0·1 Hz. *A*, instantaneous current. Mean data are shown from seven cells at age 1 day (○), from seven cells at 5 days (●) and from eight cells at 10 days (△). Data are shown with bars depicting 1 S.E. of the mean which fall within plotted points in some cases. Similar type symbols are used to depict data at different ages in all subsequent figures. The arrowed potential denotes the predicted V_K. *B*, steady-state currents recorded at the end of 400 ms clamp steps used to obtain data in *A*. 1-Day data are mean of eight cells, 5 days of seven cells, 10 days of eight cells.

These data suggest that different inactivation properties of I_{K1} at different ages may have a role in the changes in mean I_{K1} that we observed (Fig. 4*A*). We have therefore investigated the voltage-dependence of inactivation and kinetic properties of I_{K1} inactivation between the three age groups. The results are shown in Fig. 5. The

protocol of the experiment is illustrated in Fig. 5*A* using current records from the three rat myocyte ages studied. All the previous data shown (Figs 3 and 4) was collected from holding potentials of −90 mV. Here, we have altered the holding potential and then hyperpolarized the cells to a fixed test potential of −150 mV in order to measure the peak inward I_{K1}. In Fig. 5*A* data from holding potentials of

TABLE 1. Time constants of relaxation of I_{K1} calculated from fits of equation (1) to original current traces

V_m (mV)	Time constant (ms)					
	1 day		5 day		10 day	
−100	135±47·2 (6)		114±33·2 (6)		112±77·7 (7)	
−110	131±66 (8)		134±66 (8)		89·4±68·7 (10)	
	τ_1	τ_2	τ_1	τ_2	τ_1	τ_2
−120	160±48 (6)	10·1±6·8 (8)	186±15 (8)	12·6±8·7 (8)	145±47 (10)	13·0±7·7 (11)
−130	184±37 (5)	11·3±7·2 (5)	145±64 (7)	14·2±15 (8)	145±51 (8)	12·6±3·8 (8)
−140	130±40 (7)	7·45±5·4 (7)	112±24 (7)	7·15±4·6 (7)	119±54 (7)	13·1±7·0 (6)
−150	114±57 (9)	10·8±5·8 (9)	104±87 (9)	12·1±11 (9)	83·4±36 (7)	12·9±8·4 (8)

Cells were hyperpolarized for 400 ms from −90 mV to potentials indicated in column labelled V_m. Data during hyperpolarizing clamp pulses to −100 or −110 mV were best fitted by a single exponential process. At more negative test potentials data were generally better fitted by dual exponential relaxations with time constants given by τ_1 and τ_2 for each age group. All data are in ms (±1 S.D.) and adjacent bracketed numbers indicate the numbers of cells studied. Almost half of all data for 10-day cells ($n = 6$) at −150 mV were still best fitted by a single exponential process of mean time constant 47·9±15·5 ms (S.D.).

−50 and −90 mV are shown from 1-, 5- and 10-day cells. Little effect on I_{K1} of changing the holding potential from −50 to −90 mV was observed in 1-day cells. However, in 10-day cells (right panel) and to a lesser extent in 5-day cells, significant inactivation of I_{K1} occurs at a holding potential of −90 mV, compared with −50 mV. These data are summarized in the graph (Fig. 5*C*) which illustrates the voltage-dependence of inactivation of I_{K1} (fractional) recorded at −150 mV as a function of holding potential (abscissa). There is a clear shift of these relations to the right (more positive potentials) with increasing age. This has important implications for the amplitude of I_{K1} in the physiological range of potentials between −30 and −90 mV. At a potential of −90 mV there is 50% inactivation of I_{K1} in 10-day cells but only 10–20% inactivation in 1-day cells. A holding potential of −140 mV is required to produce 50% inactivation in 1-day cells (a potential that inactivates I_{K1} by more than 80% in 10-day cells). Again, 5-day cells seem to form a transitional group between the younger and older cells.

The kinetics of inactivation of I_{K1} at different ages are shown in Table 1 and graphically in Fig. 5*B*. Exponential functions were fitted to the inactivating portion of I_{K1} using the DISCRETE program (Provencher, 1976) with up to three exponential terms of the form:

$$I_{K1(t)} = A_1 \exp(-t/\tau_1) + [A_2 \exp(-t/\tau_2)] + A_0, \quad (1)$$

where A_0 is a time-independent current component, A_1 and τ_1 are the initial amplitude and time constants of a single component. The second expression within

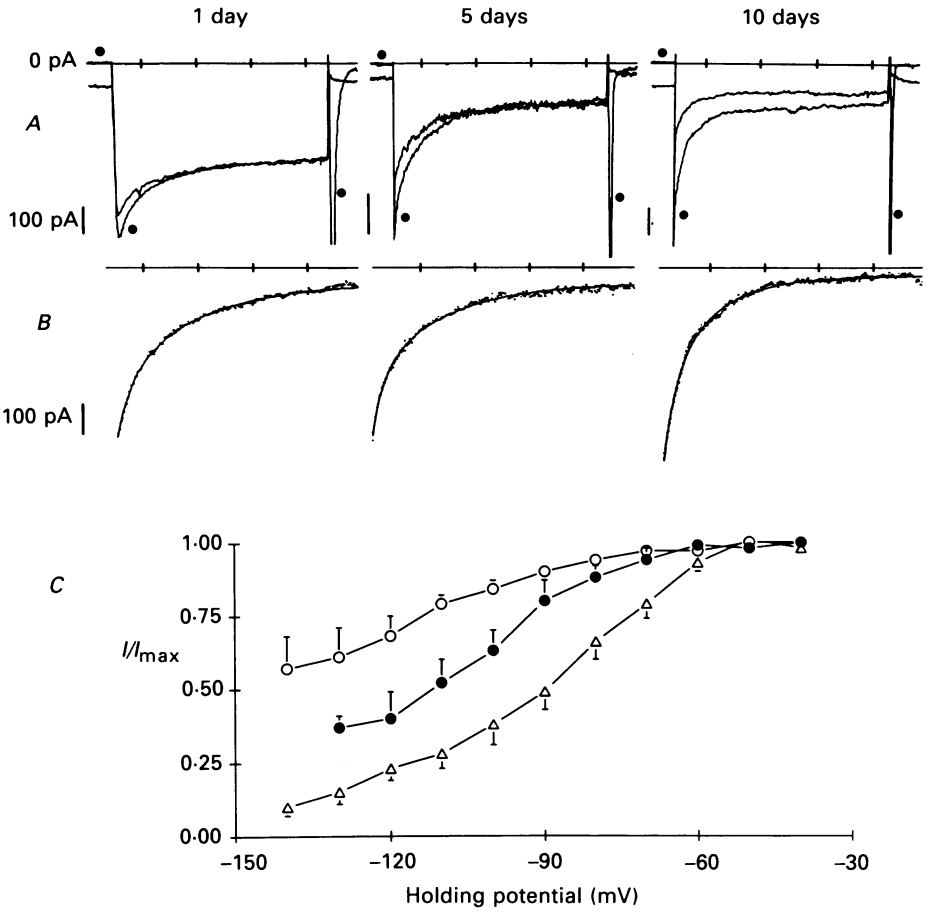

Fig. 5. Voltage-dependence of inactivation of I_{K1}. *A*, representative records of total membrane current recorded during hyperpolarizing voltage-clamp steps to −150 mV from −90 and −50 mV (traces marked with ●) at 1, 5 and 10 days of age as indicated. *B*, dual exponential fits to data in *A* from a holding potential of −50 mV. Continuous line represents curve generated from fitted parameters, dotted points are original data. Calculated time constants were 84·1 and 14·8 ms for 1-day data, 74 and 11·3 ms for 5-day and 36 and 8·4 ms for 10-day data. The time marks above each panel denote 100 ms in *A* and 60 ms in *B*. *C*, graphical analysis of effect of holding potential on availability of I_{K1} at different ages. Data collected during 400 ms hyperpolarizing voltage-clamp pulses to −150 mV from a range of holding potentials (abscissa). Peak I_{K1} (Ba^{2+}-sensitive current) at −150 mV from each holding potential is expressed as a fraction of the maximum evoked when no inactivation is present (i.e. from a holding potential of −40 mV). Data are shown ±1 S.E.M. Data from seven cells at 1 day (○), four cells at 5 days (●) and six cells at 10 days of age (△).

square brackets represents the second and subsequent faster exponential components with corresponding amplitudes and time constants. Cells were hyperpolarized to potentials between −100 and −150 mV for 400 ms from −90 mV. Generally, for small hyperpolarizations to −100 or −110 mV, data were best fitted by a single exponential process with a time constant longer than 100 ms (Table 1). At more

negative pulse potentials data were well fitted by a single exponential process, but were significantly better fitted by a dual exponential process. The twin exponentials are illustrated by the continuous lines through data in Fig. 5*B*. The time constants for data in Fig. 5*A* ranged between 7·8 ms and 14·8 ms for the fast component (τ_2) and

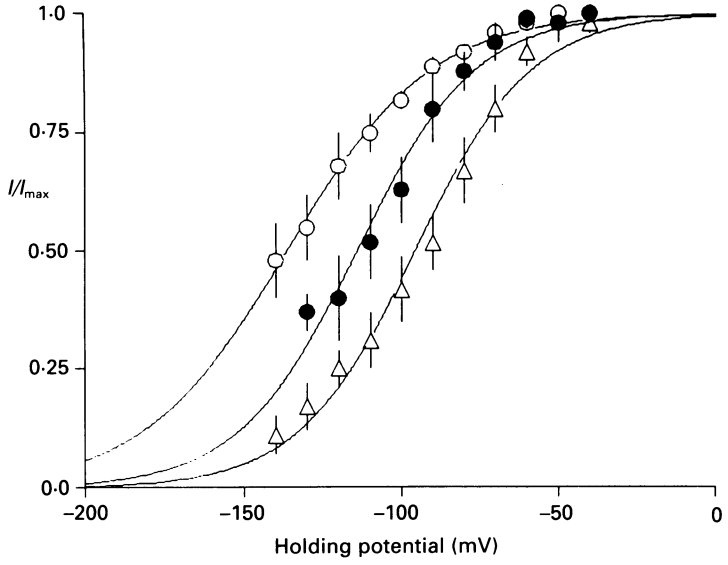

Fig. 6. Fit of data from Fig. 5*C* to Boltzmann functions. Original data of voltage-dependent inactivation of I_{K1} at different ages have been replotted on a wider potential axis. Continuous curves were generated from equation (2). The half-inactivation potentials and slope factors were −136 and +22·7 mV respectively for 1-day cells (○); −114 and +18·7 mV for 5-day cells (●) and −95·6 and +18·4 mV for 10-day cells (△). Data are shown ±S.E.M. Note that only from cells from 1-day data have been included.

36 and 95 ms for the slow component (τ_1). A noticeable trend in Table 1 between 1- and 10-day cells is the acceleration of the slower time constant with increasing developmental age, i.e. at −150 mV, τ_1 decreases from 114 ms at 1 day to 83·4 ms at 10 days of age. However, these differences were not significant at any potential studied. The principal difference between 1-, 5- and 10-day inactivation processes for I_{K1} was that the amplitude of the two exponential processes were quite different. This may be clearly seen in the data in Fig. 5*A*. The 1-day I_{K1} relaxation appears to have mainly a slow component (see also Fig. 3) whereas the 10-day current traces have primarily a fast component. We have expressed this as the relative amplitude of the fast component (A_2) divided by the total inactivating current (A_1+A_2) where A_1 is the amplitude of the slow component. There appeared to be little voltage-dependence to these ratios, but they were quite age-dependent, as expected from examination of data in Fig. 5*A*. For 1-day cells mean $A_2/(A_1+A_2)$ was 0·48±0·12 (S.D., $n = 29$); for 5-day cells mean ratio was 0·52±0·17 ($n = 35$) and for 10-day cells 0·67±0·16 ($n = 41$). The mean ratio was significantly greater for 10-day cells compared with 5-day and 1-day cells ($P < 0·001$ in both cases), but not significantly different between 1-day and 5-day cells. As stated above, (and Table 1) a twin-exponential provided the

best fit of the inactivation of I_{K1} transients, but often current relaxations during hyperpolarizing voltage-clamp steps could be adequately fitted by a single exponential relaxation, so we have attempted to describe the voltage-dependence of inactivation using a single Boltzmann function.

The potential-dependence of I_{K1} inactivation can be approximately described by a Boltzmann function (e.g. Ohmori, 1978), i.e.

$$I/I_{max} = 1/1+\exp[(V_{\frac{1}{2}}-V_m)/S], \quad (2)$$

where $V_{\frac{1}{2}}$ is the membrane potential at which half-inactivation occurs and S is the slope factor at $V_m = V_{\frac{1}{2}}$.

The data in Fig. 5*C* have been transformed and fitted curves according to equation (2) are displayed in Fig. 6. All curves have a similar slope factor $\sim +20$ mV (see legend), but are shifted to the right along the voltage axis with age from a half-inactivation potential of -136 mV for 1-day cells to $-95{\cdot}6$ mV for 10-day cells.

Transient outward currents

Data in Fig. 2 showed action potential prolongation that resulted from exposure of cells to 4-AP. In view of these data and the known presence in adult rat cells of a relatively large transient outward current (Josephson *et al.* 1984) we decided to investigate the nature and kinetics of the transient outward currents in rat ventricle as they changed during development. In Fig. 7 membrane currents are shown from a 1-day and a 10-day cell. These show, in both age groups, that there is little effect of blocking I_{Na} with $3{\cdot}1 \times 10^{-5}$ M-TTX on the measurement of the transient outward current (arrowed traces in Fig. 7*A* and *B*). Subsequent exposure of cells to 3×10^{-4} M-$CdCl_2$ to block the Ca^{2+} current, the Ca^{2+}-activated transient outward currents and other Ca^{2+}-activated K^+ currents, has little further effects on the membrane current traces (Fig. 7*C* and *D*). This indicates that the predominant transient outward current during development in rat cells is unlikely to be Ca^{2+}-activated. The result also suggests that other Ca^{2+}-activated K^+ currents do not have a major role in these cells. Subsequent exposure of cells to 2×10^{-3} M-4-AP (Fig. 7*C* and *D*) removes time-dependent outward current, suggesting that the predominant transient outward current in rat cells is 4-AP-sensitive and time- and voltage-dependent.

It is apparent from data in Fig. 7 that the nature of I_t in 1-day cells is quite different from the current in 10-day cells. At the test potentials illustrated the kinetics of I_t in 1-day cells are much slower than in the 10-day cells. Full current–voltage relations for I_t are shown in Fig. 8. The original records in Fig. 8*A* illustrate that the transient outward current appears to be larger in older cells. In many 1-day cells little or no transient outward current was observed. The numbers of cells without I_t decreased by 5 days and almost all 10-day cells possessed I_t. Again, apart from size, there appears to be differences in the kinetics of I_t between the groups. Generally the inactivation time course of I_t is delayed in the youngest age group. The graph in Fig. 8*B* summarizes current–voltage relations from rat neonatal cells. It is apparent that the current density of transient outward current increases in older cells, in a ratio of about 4:2:1 for cells aged 10, 5 and 1 day respectively.

Another feature of transient outward currents from 1-day cells was that the activation threshold for the current appeared to be displaced to more positive

potentials compared with the older cells. The data in Fig. 8*B* at potentials positive to 0 mV (where channel open probability for I_t was assumed to be almost 1·0) was fitted by a least squares method. Extrapolation of the transient outward current–voltage relation back to the zero current potential gives a voltage of

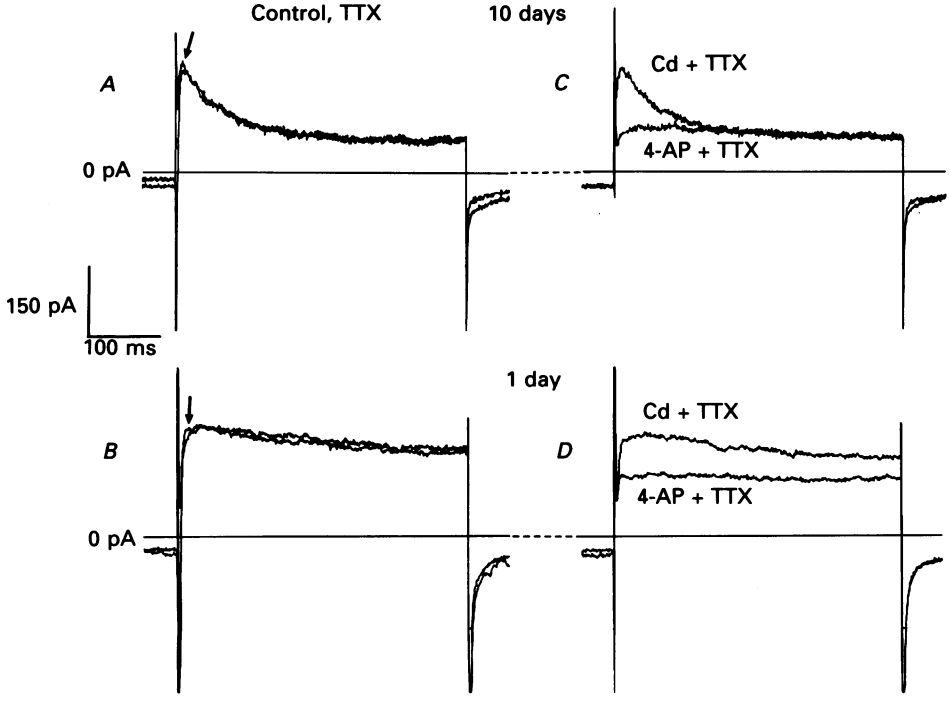

Fig. 7. Transient outward current recorded from a 1-day and a 10-day cell under the influence of various agents. *A* and *C*, 10-day cell; transient outward current during 400 ms depolarizing voltage-clamp steps from −90 to +20 mV showing control and the effect of $3{\cdot}1 \times 10^{-5}$ M-TTX (arrowed) in *A*. *C* shows subsequent effect of 3×10^{-4} M-$CdCl_2$ and 2×10^{-3} M-4-AP in the presence of TTX. *B* and *D*, 1-day cell; membrane current records during depolarizing voltage-clamp steps from −90 to +20 mV in control and TTX (arrowed) in *B* and subsequently $CdCl_2$ and 4-AP in *D*. Doses in *B* and *D* as in *A* and *C*.

−7·55 mV for 1-day cells, −22·4 mV for 5-day and −21·6 mV for 10-day cells. This result suggests that either there is a shift in I_t reversal potential to more negative potentials in 5- or 10-day cells or alternatively there may be a shift in the potential-dependence of activation of I_t to more positive potentials in the 1-day cells, which would make an extrapolation on the basis of driving force alone, incorrect.

The transient outward current kinetics and voltage-dependence are described in Figs 9 and 10. The steady-state voltage-dependence of inactivation (Fig. 9) was obtained in two ways. First, the holding potential of cells was changed while the cell was pulsed to a constant test potential for 400 ms at 0·1 Hz. After a few pulses a steady state was reached. Alternatively, in a few cells a two-pulse protocol was used (Fedida, Shimoni & Giles, 1990). In this method a 500 ms prepulse to a range of potentials was followed by a 400 ms test pulse to a fixed potential to elicit I_t. These paired pulses were delivered at 0·1 Hz from a holding potential of −90 mV. In Fig.

9*A* steady-state inactivation relationships are shown for 1-, 5- and 10-day cells. Currents obtained from different holding potentials (or prepulse potentials) have been normalized to the maximum current obtained at the most negative potentials studied. These data were difficult to obtain from 1- and 5-day cells in which rather

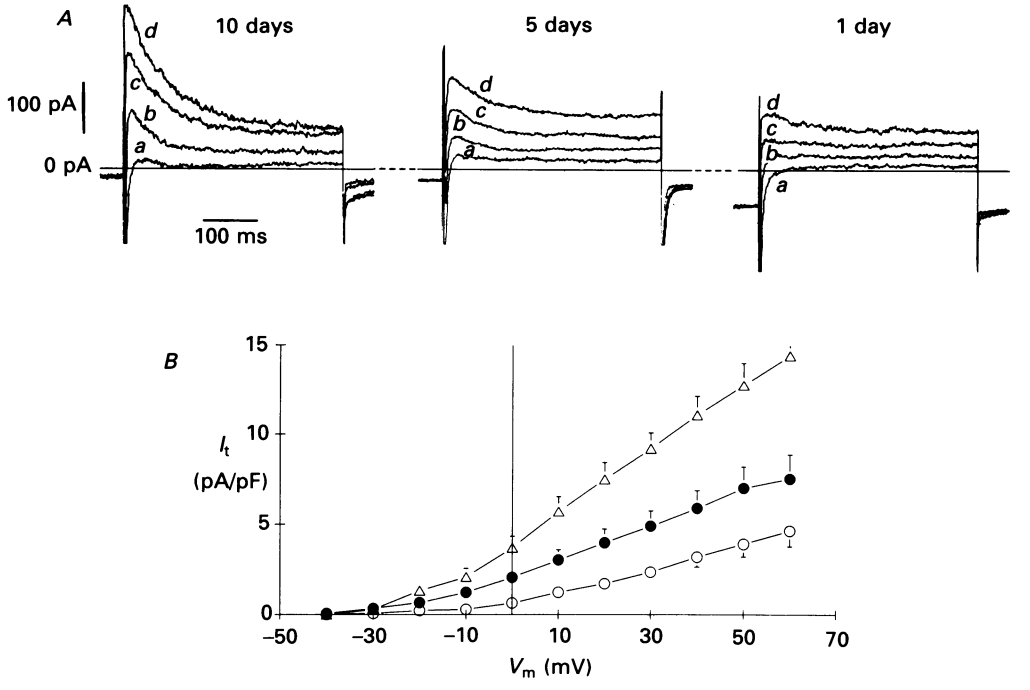

Fig. 8. Peak current–voltage relations of transient outward current recorded from cells of different ages. *A*, representative current records from a 10-, 5- and 1-day cell. Cell capacitances 15·3 pF (10 days), 24 pF (5 days) and 8·9 pF (1 day). Voltage-clamp pulses were given for 400 ms from a holding potential of −90 to −20 mV (*a*), +10 mV (*b*), +20 mV (*c*) and +40 mV (*d*). 3×10^{-4} M-$CdCl_2$ present in all cases. *B*, cumulated *I–V* relations from sixteen cells at 1 day of age (○), sixteen cells at 5 days (●) and eighteen cells at 10 days (△). All data normalized to cell capacitance and expressed ±1 S.E.M. Note the increase in I_t with developmental age and the lack of significant I_t negative to 0 mV in 1-day cells.

positive test potentials had to be used in some cells to elicit sufficient I_t for measurement. However, the data from all three age groups fall on similar sigmoidal curves. Inactivation is almost entirely removed for holding potentials negative to −90 mV and is nearly complete for potentials positive to −30 mV. For comparison with adult cell data we have combined all data from our experiments to produce a single curve and have fitted the data to a curve given by equation (2). The data and calculated curve are shown in Fig. 9*B* where the half-inactivation potential ($V_{\frac{1}{2}}$) of −59·9 mV was obtained where I/I_{max} was 0·5.

The data in Fig. 10 illustrate the changes in the fully activated current–voltage relations during development from 1 to 10 days of age. It is well known that deactivation of I_t can be extremely rapid on repolarization to potentials more

negative than 0 mV, but this is less of a problem in the small spherical cells with capacitances around 10 pF. In this case the predicted membrane time constants of 50–100 μs are not likely to obscure I_t tails, nor are special methods therefore required to cancel the capacity transient. From a holding potential of −90 mV cells were

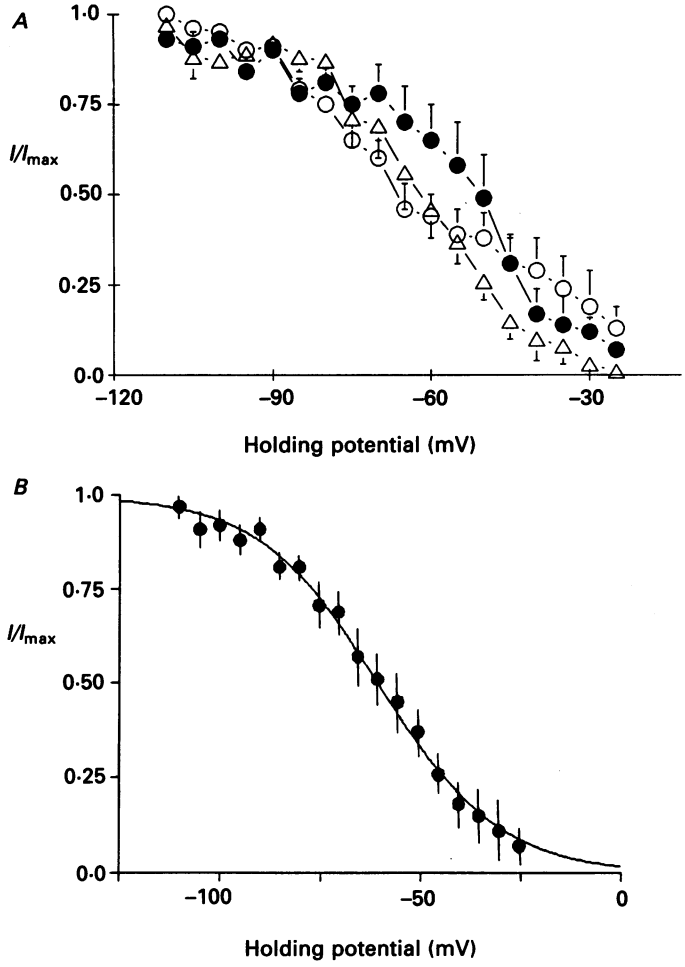

Fig. 9. Steady-state voltage-dependence of inactivation of I_t in cells of different ages. Data was obtained by normalizing I_t to maximum recorded during voltage-clamp steps to a constant test potential from a range of holding potentials. In total relations were obtained from four 1-day cells (○), five 5-day cells (●) and seven 10-day cells (△). In two of the 10-day cells and two of the 5-day cells currents were measured using a two-step clamp protocol (see text). *B*, fit of data in *A* to a Boltzmann function as shown in equation (2). Data are shown ±1 S.E.M. Continuous line fitted from the equation with a slope factor of −15·1 mV and a half-inactivation potential of −59·9 mV.

briefly depolarized (10–20 ms) to a potential that allowed complete activation of I_t (~ +40 mV). Before significant inactivation could occur cells were subsequently repolarized to a range of potentials to record tails of transient outward current. The principal aim of this experiment was to make a measurement of the reversal potential

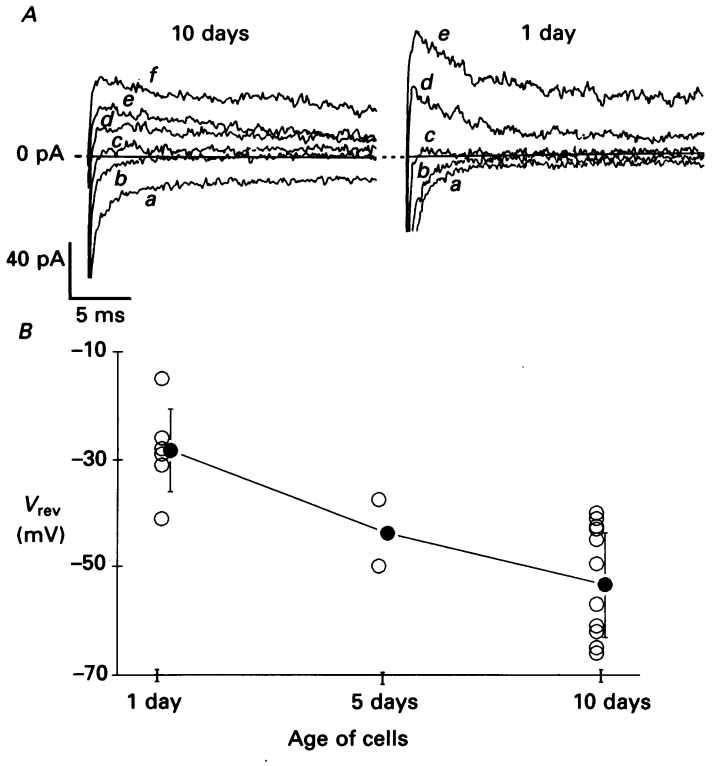

Fig. 10. Deactivation and reversal potential of I_t. *A*, representative current records from a 10-day and a 1-day cell as indicated. Voltage protocol was that cells were held at −90 mV, briefly depolarized (10–20 ms) to a potential sufficiently positive to fully activate I_t (usually +40 or +50 mV). Cells were then immediately repolarized to a range of potentials from +30 to −80 mV to look for the reversal of the current tail. In *A* only the current traces during the repolarizing test steps are shown. In the 10-day cell data, repolarization potentials shown were (mV): *a*, −80; *b*, −70; *c*, −60; *d*, −50; *e*, −40; *f*, −30. For the 1-day cell the potentials were (mV): *a*; −55; *b*, −45; *c*, −35; *d*, −25; *e*, −15. Note that the tail currents appear to reverse in direction at ~ −60 mV in the 10-day cell and at ~ −35 mV in the 1-day cell. *B*, cumulated data on the reversal potential of I_t in cells of different ages. Reversal potential (V_{rev}) is plotted on the ordinate, increasing negative downwards. Six cells at 1 day, two cells at 5 days and thirteen cells at 10 days were studied. Current tail amplitudes were measured from traces such as shown in *A*. The reversal potential was estimated from a graph of these values for each cell. Mean reversal potentials for I_t at different ages are shown as the filled circles in *B* (values ±1 S.D.). There is a significant negative shift of the reversal potential from −28·3 mV in newborn cells to −53·4 mV at 10-days of age ($P < 0.001$, Student's *t* test).

for I_t. Representative current records obtained during the repolarizing voltage steps from 1- and 10-day cells are shown in Fig. 10*A*. In the 10-day cells repolarization to −30 or −40 mV elicits a decaying outward tail current after the capacity transient. This appears to reverse in direction at about −60 mV. The 1-day data are rather different. Here, the current reversal seems to occur near −35 mV. Data from experiments such as those shown in Fig. 10*A* were plotted to obtain a graphical estimate for the reversal potential of I_t (V_{rev}). Cumulated V_{rev} values from the three

age-groups of cells are shown in Fig. 10*B* along with mean values (filled symbols). There is a significant shift of the reversal potential for I_t in the hyperpolarizing direction with increasing age. In addition, in five 10-day cells we have obtained fully activated current–voltage relations in both 5×10^{-3} M-K^+ and 2×10^{-2} M-external K^+. The shift in V_{rev} amounted to $+21{\cdot}4 \pm 4{\cdot}4$ mV (mean ± S.D.). For three 1-day cells the mean shift amounted to $+15{\cdot}3$ mV. All the V_{rev} values obtained for I_t and the shifts of these values in the depolarizing direction in high external K^+ are rather less than expected for highly selective K^+ currents. The calculated potassium equilibrium potential (V_K) was -85 mV and the expected shift for a change in external K^+ from 5×10^{-3} to 2×10^{-2} M is $+35$ mV. These results indicate that the I_t channel in rat neonatal cells has a rather poor selectivity for K^+ at birth but that the selectivity for K^+ increases somewhat by 10 days of age.

DISCUSSION

Developmental changes in rat neonatal action potentials

Our results show that a number of wide-ranging changes occur in the action potential of rat heart in the first 10 days after birth. This should not be surprising as fundamental ultrastructural changes are occurring in these cells both prenatally (Couch *et al.* 1969; Bernard, 1975) and in the immediate postnatal period (Legato, 1972). At birth the myofibrils are sparse, the sarcoplasmic reticulum is poorly developed and the T-tubular system is absent (Legato, 1972: Hirakow & Gotoh, 1975; Hoerter, Mazet & Vassort, 1981; Cohen & Lederer, 1988). During the first week there is continuous development of the SR, T-tubular system (Legato, 1972; Moses & Kasten, 1979) and maturation of the mitochondria begins to occur. By 14 days all ultrastructural elements are at a relatively advanced stage (Hirakow & Gotoh, 1975). Functionally, the sarcoplasmic reticulum is suggested to have only rudimentary function (Seguchi, Harding & Jarmakani, 1986; Cohen & Lederer, 1988) and mechanically Hopkins, McCutcheon & Wekstein (1973) suggest that maturity is complete at about two weeks after birth. The resting potentials that we have recorded from these cells are in the range -60 to -70 mV (Figs 1 and 2). These values are comparable with those recorded by Robinson (1982) who described no change in the maximum diastolic potential of neonatal cells in the perinatal period, with a mean value of the resting potential of ~ -70 mV. The action potentials that we have recorded from neonatal rat ventricular cells are very similar to those recorded from intact tissue. At 1 day most authors have reported that action potentials have a prolonged plateau phase (Couch *et al.* 1969; Langer *et al.* 1975; Jourdan & Sperelakis, 1980). It is not entirely clear what the true plateau amplitude is in newborn cells. In the majority of our impalements (Figs 1 and 2; Brown *et al.* 1986) there is still a definite 'notch' denoting an early phase of rapid repolarization. This depression of the plateau to around 0 mV is also apparent in the single myocyte data of Cohen & Lederer (1988, Fig. 1) and has been observed in action potentials recorded from multicellular tissue by Langer *et al.* (1975). However, others have reported neonatal action potentials with action potential plateaux around $+20$ mV (Couch *et al.* 1969; Pučelík *et al.* 1982, 1983). One explanation of this plateau depression might be the fact that the present experiments were carried out at room

temperature, as were the experiments of Cohen & Lederer (1988). The calcium current is known to have a high Q_{10} (Cavalié, McDonald, Pelzer & Trautwein, 1985) and a relative reduction of this current compared with repolarizing K^+ currents might be the cause of the lower plateau phase. Alternatively, significant heterogeneity has been reported for individual myocytes and strips of tissue isolated from rat ventricular myocardium (Watanabe, Delbridge, Bustamante & McDonald, 1983). Our data also shows heterogeneity of action potential shape for the population of cells studied within each age group (compare 10-day action potentials in Figs 1 and 2). This may reflect a heterogeneity of the mix of ionic currents present in individual cells. Such a heterogeneity exists in adult canine ventricle, especially between endocardium and epicardium (Litovsky & Antzelevitch, 1988), but also regionally and in the newborn (Spach, Dolber & Anderson, 1989). In developing rat myocardium this may also be the case (Jourdan & Sperelaksi, 1980), but nonetheless, action potentials recorded from our single myocytes are very similar to those which have been recorded from multicellular tissues over the past 20 years, and show similar age-dependent changes (Langer *et al.* 1975; Půcelík *et al.* 1982). Hence these cells appear appropriate for quantitative studies of the ionic basis of changes in action potential shape.

Transient outward current

There is a progressive shortening of the newborn rat action potential in the period up to 10 days of age. At this time the adult form has still not been reached, the duration of which is often less than 100 ms (Figs 1 and 2, Josephson *et al.* 1984). 4-Aminopyridine has small effects on the early phase of repolarization of newborn rat action potentials (Fig. 2) which suggests that voltage-activated transient outward current is of little functional importance in these cells. In contrast, 4-AP has more pronounced effects on the action potentials of 5- and 10-day cells and a marked prolongation occurs in adult cells exposed to the drug. In keeping with these changes there are major developmental changes in the properties of I_t in these cells. I_t is often absent, or apparent only at positive potentials in newborn cells (Fig. 8) and there is an approximately four-fold increase in the current density of I_t from 1- to 10-day cells. This increase in I_t density is recorded despite an increase in mean cell capacitance of only 1·8 over a similar time period. It is not clear how much of the increase in cell capacitance reflects increases in sarcolemmal membrane as this period is thought to be an important one for both the development of SR membrane (Seguchi *et al.* 1986; Cohen & Lederer, 1988) and T-tubules (Hirakow & Gotoh, 1975; Moses & Kasten, 1979). Adult rat ventricular cells have a high individual capacitance of around 200 pF (Powell *et al.* 1980) and in these cells there is a very large transient outward current that can be many nA in a single cell (Josephson *et al.* 1984). Developmental changes in the human atrial and rabbit ventricular (Saxon & Safronova, 1982) action potential are very similar to those described here for the rat. There is the development of a rapid initial notch on repolarization with age that depresses the action potential plateau to negative potentials. This effect is rate-dependent and sensitive to 5×10^{-4} M-4-AP so has been attributed to the increase in magnitude of I_t with age in humans (Escande, Loisance, Planche & Coraboeuf, 1985).

The reasons for the increase in I_t with developmental age were investigated by

recording developmental changes in the nature and kinetics of I_t (Figs 7–10). The transient outward current was found to be of the voltage-activated type in newborn cells and throughout development. Josephson *et al.* (1984) have previously shown that adult rat transient outward current is predominantly of the time and voltage-dependent type. Experiments to test for a Ca^{2+}-activated K^+ current, either transient in nature (Siegelbaum & Tsien, 1980) or time independent $I_{K(Ca)}$ revealed that no Cd^{2+}-sensitive current component could be found (Fig. 7). No change in the steady-state inactivation properties of transient outward current was observed during development (Fig. 9). The steady-state inactivation relation was well fitted by a single Boltzmann distribution with a half-inactivation potential of −60 mV. This value is quite similar to the −63 mV described by Josephson *et al.* (1984) in adult rat cells, although the slope factor was shallower in our experiments. This means that inactivation develops over a greater potential range, 75 mV compared with 30 mV. The reason for this may simply be the protocol used to measure study-state inactivation. Fedida *et al.* (1990) have shown in rabbit atrial cells that a holding potential method to assess steady-state inactivation results in a relation with a broader voltage-dependence than those generated using a two-pulse protocol. The experiments of Josephson *et al.* (1984) were carried out using a two-pulse protocol. Our results are then in agreement with adult data on this point. We did, however, find developmental changes in the reversal potential of the transient outward current in these cells. Extrapolation of the current–voltage relations back to the zero current potential indicated that the reversal potential in newborn cells might be more positive than the reversal potential of I_t in 10-day cells (Fig. 8). The mean reversal potential for 10-day cells was −53·4 mV, 25 mV negative to the reversal potential in newborn cells (Fig. 10) and the effect of increasing external K^+ was also greater in the older cells, all of which suggests that the selectivity for K^+ of the transient outward current is present in all young cells studied, but that it is least in newborn cells. This relatively positive V_{rev} for I_t in 1-day cells may well be the reason that so few cells possess measurable I_t at potentials between −30 and 0 mV (Fig. 8). This finding provides an explanation for the lack of 4-AP on the early phase of repolarization of the action potential of newborn cells and underlines the lack of importance of I_t in the physiological range of potentials in these cells at this stage of development.

The inward rectifier

We have used a step voltage-clamp protocol from a holding potential of −90 mV and $BaCl_2$ subtraction of currents to obtain a measure of the background K^+ currents during development in rat neonatal cells (Figs 3–6). On the basis of its reversal potential near V_K, the strong inward rectification, the presence of inactivation at negative test potentials (Fig. 3) and the presence of a region of negative slope conductance we attribute this macroscopic current to the presence of I_{K1} channels. To our surprise we have demonstrated a marked developmental decrease in the amount of available I_{K1} current density during development from the newborn to 10 days of age. This is in the light of an increase in mean cell capacitance over this time period. It is interesting that in chick ventricular myocytes a decrease in background current occurs from 7 to 17 days of embryonic development (Clay & Shrier, 1981) and a decrease in I_{K1} from 4 to 7 days postnatally has been reported (Satin & De Haan,

1988). The membrane potentials at which this decrease is likely to be of the greatest importance are the last 30–40 mV of repolarization, i.e. between −30 and −70 mV. The I_{K1} relations show a significant 'hump' of outward current in 1- and 5-day cells in this voltage range, whereas in 10-day cells the relation is quite flat. This outward current will tend to accelerate the final phase of repolarization in newborn cells (Figs 1 and 2) and accentuate the similarity in action potential shape between these cells and other mammalian action potentials from guinea-pig or rabbit (Giles & Imaizumi, 1988). In contrast the older tissue can be expected to have a tendency to repolarize more slowly over this range of potentials because of the smaller I_{K1}. One consequence of this might be the ability of inward currents activated at negative potentials to affect the final phase of repolarization of the action potential, as has been shown for sodium–calcium exchange current in adult rat ventricular cells (Schouten & ter Keurs, 1985).

Part of the reason for the decrease in I_{K1} in the older neonatal cells may be due to changes in their voltage-dependent or ionic block-dependent properties. Prominent relaxations of I_{K1} were recorded at negative membrane potentials as previously seen in a number of single cell preparations (Figs 3–5; e.g. Sakmann & Trube, 1984). Inactivation of I_{K1} has been attributed to external Na^+ ions in frog skeletal muscle (Hille & Schwarz, 1978), in tunicate egg cell membranes (Ohmori, 1978) and, in combination with external or internal cations such as Ca^{2+} and Mg^{2+}, in guinea-pig ventricular myocytes (Biermans, Vereecke & Carmeliet, 1987; Matsuda, Saigusa & Irisawa, 1987). In neonatal (1- to 3-day) rat ventricular cells Payet, Rousseau & Sauvé (1985) and Josephson & Brown (1986) have recorded whole-cell I_{K1} and find similar current relaxations to those we have described in the youngest cells (Figs 3 and 5; Fig. 1*Aa* and *B*, Josephson & Brown, 1986). It is interesting that in the experiments of Josephson & Brown (1986), external Na^+ ion removal failed to prevent I_{K1} inactivation and they concluded that although Na^+ could act as a modulator of the kinetics of the inward rectifier (Hagiwara & Yoshii, 1979), inactivation during pulses to hyperpolarized potentials resulted from an intrinsic change in the voltage-dependent probability of opening of the inward rectifier channel. In our experiments there were significant changes in the kinetic properties of I_{K1} during development from 1 to 10 days of age. From a holding potential of −90 mV inactivation of I_{K1} during hyperpolarizing steps was less in 1-day cells than in older cells (Figs 3, 4*B* and 5*A*). Investigation of the inactivation properties of I_{K1} (Figs 5 and 6) revealed that the older cells showed a shift in the voltage-dependence of inactivation to more positive membrane potentials (Fig. 5*C*). Our data do not test whether the inactivation properties of I_{K1} arise from an intrinsic voltage-dependent mechanism for I_{K1} inactivation or differences in the age-dependence of cation-induced inactivation of I_{K1} (Sakmann & Trube, 1984; Biermans *et al.* 1987). However, this shift may have some physiological significance for I_{K1} in these cells. At a resting potential of ~ -80 mV, I_{K1} can be almost 40% inactivated in the older cells when newborn cells show less than 10% inactivation. As the *I–V* relations for I_{K1} (Fig. 4) were generated from a holding potential of −90 mV much of the apparent reduction in I_{K1} described to occur with age may arise from differences in the voltage-dependence of inactivation of I_{K1} (Figs 5 and 6). As I_{K1} was shown to inactivate in a potential range around cell resting potentials, it is interesting to speculate that

pacemaker activity observed in rat neonatal cells which have been developing in culture for some days may reflect in part a contribution from a time-dependent inactivation of inward rectifier current (Hirano & Hiraoka, 1988).

The relaxations of I_{K1} during hyperpolarizing voltage-clamp steps (Fig. 5*A* and *B*) could be well fitted by both single and dual exponential processes, so a single Boltzmann function was fitted to data from each age group (Fig. 6). We did not collect data at more negative potentials than −150 mV to avoid damage to cells and so the 1-day curve does not have points more negative than $V_{\frac{1}{2}}$. However, the fit to data predicts almost complete inactivation of I_{K1} at potentials between −200 and −220 mV, which is in agreement with published whole-cell data (Payet *et al.* 1985; Josephson & Brown, 1986). The constants determining the maximum slopes of the curves in Fig. 6 (S in equation (2)) are similar ($\sim +20$ mV, see legend to Fig. 6) and equivalent to other reports on the inactivation kinetics of the inward rectifier (e.g. in tunicate eggs, $S = +19$ to $+22$ mV; Ohmori, 1978). From these values we can calculate the effective valence of the blocking reaction, z', given the assumption that the block of current in the inward direction is caused by monovalent cations in a voltage-dependent manner (Hille & Schwarz, 1978). At 22 °C z' is 1·11 for 1-day, 1·35 for 5-day and 1·39 for 10-day cells. We interpret the similarity between these values to mean that there is no significant structural alteration to the channel structure or the gating moiety of the channel during the first ten days of life in the rat. The values of z' are rather similar to those observed for external Cs^+ ion block of inward current through inward rectifier channels in starfish eggs and squid giant axon ($z' = 1{\cdot}3$–$1{\cdot}5$; cf. Hille & Schwarz, 1978). It is interesting that in tunicate eggs, external Na^+ ions were thought to be the main external blocking cation for inward current through inward rectifier channels, as has also been suggested in cardiac tissue (Biermans *et al.* 1987). Clearly further experiments are required to elucidate the exact mechanisms of I_{K1} inactivation at negative membrane potentials at different developmental ages in rat ventricular myocytes.

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