The Journal of Neuroscience, June 1992, 12(6): 2007–2014

Expression of the H-*ras* Oncogene Induces Potassium Conductance and Neuron-specific Potassium Channel mRNAs in the AtT20 Cell Line

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Expression of the EJ-*ras* oncogene in the AtT20 cell line results in several changes in their properties that correspond to a switch of these anterior pituitary-derived cells to a more neuronlike phenotype. The width of action potentials following transfection with *ras* is reduced 20-fold from over 200 msec in control AtT20 cells to less than 10 msec in *ras*-transfected cells. This is associated with a two- to threefold increase in the density of voltage-dependent potassium currents. In addition, the rate of inactivation of these currents is decreased approximately twofold in *ras*-transfected cells. At least part of the change in potassium current may be due to differential expression of potassium channel mRNAs. In the *ras*-transfected cells, mRNA species were detected using a probe for the voltage-dependent potassium channels, Kv4, a species that appears to be uniquely expressed in the nervous system, and NGK2, an alternatively spliced product transcribed from the same gene. These mRNAs are not detected in control AtT20 cells. The results suggest that the *ras* protein modulates the phenotype of excitable cells by influencing the expression of specific potassium channels and thereby altering the density and types of channels in the plasma membrane.

The *ras*-family of protooncogenes encodes a set of 21 kDa proteins ($p21^{ras}$) and is a member of a GTPase superfamily of genes whose products bind and hydrolyze guanine nucleotides (for a recent review, see Bourne et al., 1990). One interesting aspect of these proteins is that they are highly expressed in neurons and neurosecretory cells (Mizoguchi et al., 1989). By analogy with other, better understood, guanine nucleotide–binding proteins that link neurotransmitter and hormone receptors to second messenger pathways, it has been suggested that the *ras* proteins participate in as yet uncharacterized signal transduction pathways (Barbacid, 1987).

The high levels of *ras* expression in the nervous system and other excitable tissues suggest that $p21^{ras}$ could be an important component of pathways that regulate the long-term excitability of neurons and other cells. To test this possibility, we have transfected the human H-*ras* oncogene EJ-*ras* (Shih and Weinberg, 1982) into the mouse neurosecretory line AtT20. The EJ-*ras* oncogene encodes a protein that differs from the normal *ras* protein by one amino acid. This lowers the intrinsic rate of GTP hydrolysis by $p21^{ras}$ and also eliminates its sensitivity to stimulation by GAP, the GTPase activator protein for $p21^{ras}$ (Vogel et al., 1988). This mutation therefore increases the fraction of time spent by the protein in the active, GTP-bound state, and by analogy with other GTP-binding proteins, fixes the protein in its active "signaling" mode.

The AtT20 cell line is derived from a pituitary tumor that secretes ACTH and β-endorphin. It has long served as a model system for the study of hormonal regulation of neuropeptide synthesis, posttranslational processing, and release (Herbert et al., 1978; Roberts et al., 1978; Burgess and Kelley, 1987). In addition, AtT20 cells are excitable and the ionic currents that underlie their electrical behavior have been partially characterized (Suprenant, 1982; Adler et al., 1983; Flamm et al., 1990). We have found that transfection of these cells with the H-*ras* oncogene induces several changes in their excitability. These include a decrease in the width of spontaneous and evoked action potentials and a change in the amplitude and kinetics of their voltage-dependent potassium currents. These changes are associated with the induction of mRNA species that encode components of voltage-activated potassium channels specific to the nervous system.

Materials and Methods

Cell culture. AtT20 cells, both normal and *ras* transformed, were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum or 10% horse serum and 2.5% fetal bovine serum. *ras*-transformed AtT20 cells were maintained in selection with 400 μg/ml G418. Details of the transfection by EJ-*ras* are described elsewhere (Birnberg et al., 1992).

Electrophysiology. Whole-cell voltage-clamp recordings were made at 21°C using an L/M EPC-7 patch clamp (List Electronics, Darmstadt-Eberstadt). Electrodes had a resistance of 1.5–2.5 MΩ after fire polishing. The tips of patch electrodes were dipped in Sigmacote (Sigma Chemical Co., St. Louis, MO) to reduce electrode capacitance prior to use. The whole-cell patch configuration was attained using either standard techniques (Hamill et al., 1981) or the perforated patch technique (Horn and Marty, 1986). Cell capacitance and series resistance were electronically compensated using the compensation controls of an EPC-7 patch clamp, which were also used to estimate cell capacitance. Data were collected using a computer (Indec Systems, Inc., Sunnyvale, CA). The external saline contained (in mM) 150 *N*-methyl glucamine, 5.4 KCl, 0.1 EGTA, 10 HEPES acid, 2.8 $MgCl_2$, and 20 glucose, pH 7.4 (with HCl). The dialyzed cell whole-cell patch experiments used an internal solution containing (in mM) 70 KCl, 70 potassium aspartate, 1 $CaCl_2$, 11 EGTA, 10 HEPES acid, and 5 Mg-ATP. The electrode solution for

Received Sept. 25, 1991; revised Nov. 27, 1991; accepted Dec. 11, 1991.

This work was supported by a PHS training grant to L.M.H., a PHS fellowship to T.M.P., a grant from Bristol-Myers Squibb to N.C.B., and NIH Grant HL38156 to L.K.K. We thank Dr. Edwin Levitan for helpful discussions and Mr. Carl Richmond for help in the preparation of the manuscript.

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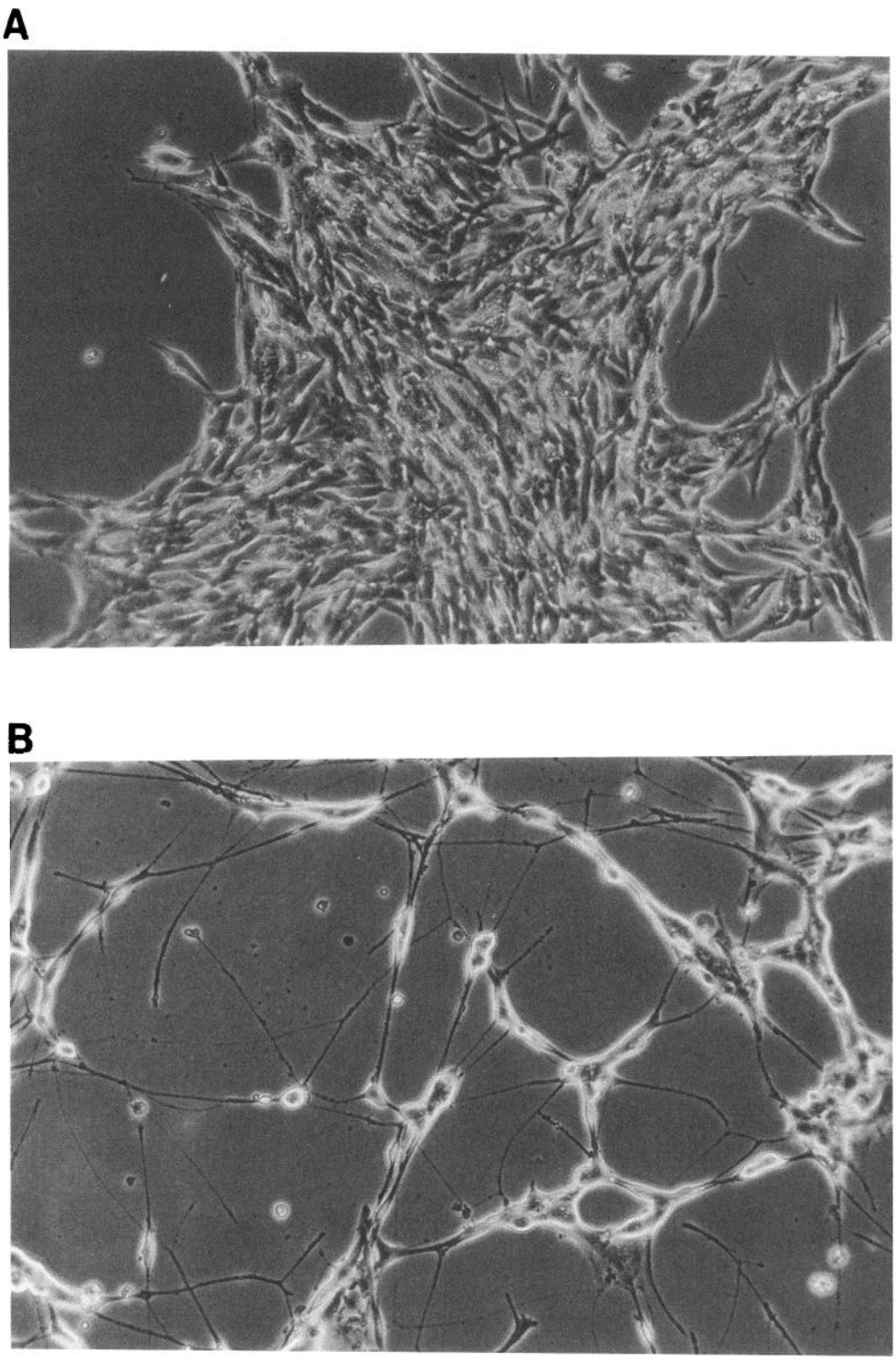

Figure 1. Phase-contrast photomicrograph of normal AtT20 cells (*A*) and EJ-*ras* transfected clone R1 (*B*).

perforated patch experiments contained (in mM) 120 K_2SO_4, 16 KCl, 5 $MgSO_4$, and 10 Na-HEPES.

Preparation of RNA and Northern blots. RNA was prepared by acid guanidinium thiocyanate–phenol–chloroform extraction, as described by Chomczynski and Sacchi (1987). PolyA RNA was obtained by elution from an oligo(dT)-cellulose column. RNA was fractionated on 0.66 M formaldehyde 1% agarose (w/v) gels in 1× morpholinepropanesulfonic acid buffer (Davis et al., 1986) with recirculation. Gels were transferred to GeneScreen Plus membranes (Du Pont New England Nuclear) in 10× saline–sodium citrate (SSC; 1.5 M NaCl, 0.15 M Na citrate). Membranes were then baked 2 hr at 80°C and prehybridized in 40% formamide, 10% dextran sulfate (w/v), 4× SSC, 7 mM Tris, pH 7.6, 1× Denhardt's solution, 1% SDS (w/v), and 200 μg/ml denatured salmon sperm DNA, at 44°C for >1 hr. A DNA fragment subcloned in pGEM-A (Swanson et al., 1990) corresponding to positions 1–1793, relative to the translation initiation codon (Luneau et al., 1991), produced by digestion with EcoRI and HindIII, was gel purified and labeled by random primer extension with *Escherichia coli* DNA polymerase I large fragment in the presence of α-^{32}P-dCTP (3000 Ci/mmol; Amersham). The sequence of the cDNA probe is unique to the Kv4 gene according to a

search of the GenBank database. This radiolabeled fragment was used as the probe for the Kv4 Northern blot. The cDNA probe for Kv1 was from positions −221 to 1908 relative to the start of translation (Swanson et al., 1990). The probe for drk1, a 750 base pair cDNA fragment corresponding to −98 to +633 relative to the initiation codon (Frech et al., 1989), was a gift from J. Trimmer. Hybridization was carried out at 44°C for 16 hr in prehybridization solution with 100 μg/ml denatured salmon sperm DNA, and 2 × 10^6 cpm/ml of radiolabeled probe. The blot was washed in 2× SSC, 0.1% SDS twice at room temperature for 15 min followed by a high-stringency wash for 30 min in 0.1× SSC, 1% SDS at 44°C. Autoradiography was performed using Kodak X-Omat AR x-ray film to visualize the hybridized probe.

Ribonuclease protection assay. Total RNA was used in the ribonuclease protection assay as described by Ausubel et al. (1990). An α-^{32}P-CTP-labeled antisense RNA probe (413 nucleotides long) derived from the 3′ end of the same Kv4 cDNA clone used in the Northern blot analysis was transcribed with SP6 RNA polymerase after linearization with PvuII. This probe is complementary to the last 398 nucleotides of the 3′ end of Kv4 and extends upstream 108 nucleotides past the splice junction for NGK2 (Luneau et al., 1991). Thus, a 108 base pair stretch of the probe is common to both Kv4 and NGK2 RNAs. An excess of the ^{32}P-labeled probe was hybridized overnight with total and polyA RNAs in 80% formamide at 45°C. Single-stranded RNA was then digested by the addition of RNases A and T1. After proteinase K treatment and phenol–chloroform extraction, the samples were precipitated with ethanol and then separated on a 4% polyacrylamide, 7 M urea sequencing gel. The protected bands were visualized by autoradiography.

Results

ras *induces changes in electrical excitability*

Cells of the AtT20/D16v subline were cotransfected with pEJ6.6, the cloned human H-*ras* oncogene (Shih and Weinberg, 1982), and the selectable marker pRSVNeo. A small number of G418-resistant colonies (three from 6 × 10^6 cells) survived selection. In contrast to normal AtT20 cells, which have a spindlelike morphology, the transfected colonies had a neuronal-like morphology with larger, typically bipolar, cell bodies and with several long neurites. Photomicrographs of normal AtT20 cells and *ras*-transfected clone R1 used in these studies are presented in Figure 1. This clone expressed the EJ-*ras* allele containing a point mutation at codon 12 and had elevated levels of the $p21^{ras}$ protein (Birnberg et al., 1992). Details of the transfection, screening, and selection of EJ-*ras* transformed AtT20 cells is described elsewhere (Birnberg et al., 1992).

Recordings of electrical activity of control AtT20 cells, made using either the whole-cell patch configuration (Hamill et al., 1981) or the perforated patch technique (Horn and Marty, 1986), showed a pattern of electrical activity that closely matched that previously reported using microelectrode recording (Adler et al., 1983). These cells generate spontaneous action potentials at a frequency of ~0.4–2.0 Hz (Fig. 2, upper trace). The mean membrane potential measured between action potentials using the whole-cell patch configuration was −54.6 ± 3.5 (SE) mV (n = 10). The action potentials are typical of neuroendocrine cells, having widths of 200–400 msec (Fig. 2, upper trace). The mean membrane potential of *ras*-transfected cells, −62.0 ± 1.8 mV (n = 17), was not very different from that of the control AtT20 cells. In all the *ras*-transfected cells, however, the duration of action potentials was substantially reduced relative to the control cells, typically lasting only about 10 msec or less (Fig. 2).

The changes in the action potentials were observed in all three *ras*-transfected clones and suggest one or more voltage-dependent ion currents expressed in *ras* oncogene-transfected AtT20 cells differ from those in control AtT20 cells. Further, in the majority of *ras*-transfected cells recorded using the conventional

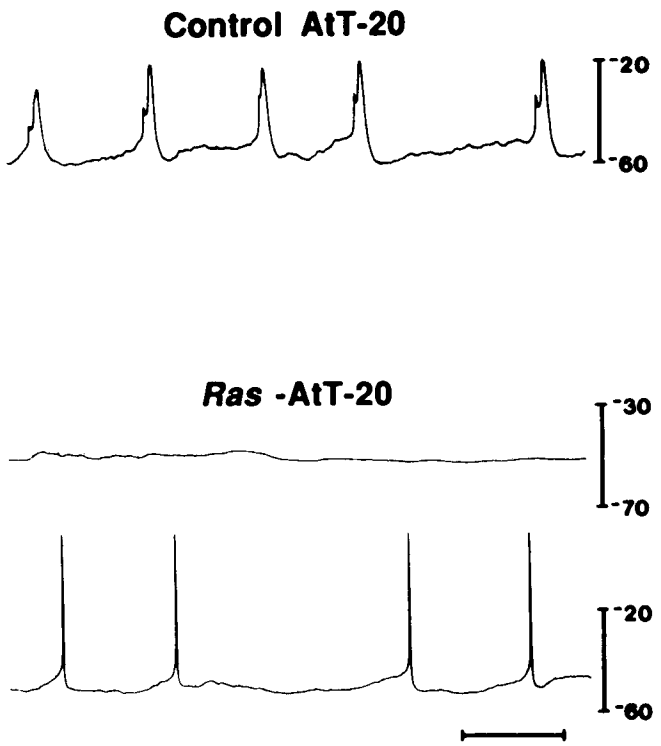

Figure 2. Action potentials recorded in a control pRSVneo-transfected AtT20 cell and *ras*-transfected clone R1. Action potentials did not occur spontaneously in this *ras*-transfected cell (*middle trace*) but could be evoked by a sustained depolarizing current (*bottom trace*). Horizontal calibration, 1 sec.

patch-clamp technique (15 of 17), repetitive activity was not spontaneous (Fig. 2, middle trace) but could only be evoked by the application of a sustained depolarizing current (Fig. 2, lower trace). In four of four *ras*-transfected cells recorded with the less invasive perforated patch technique, however, spontaneous firing of normal action potentials such as in Figure 2 (bottom trace) was observed in the absence of applied current. We speculate that the perforated patch technique may preserve any diffusible components that affect spontaneous activity to a greater extent than conventional whole-cell dialysis.

Because voltage-dependent potassium currents are an important determinant of action potential width, we analyzed these currents in detail in normal AtT20 cells and in one of the *ras*-transfected AtT20 clones (R1). Figure 3*A* shows whole-cell patch-clamp recordings of outward currents from a normal cell on the left and a *ras*-transfected AtT20 cell on the right. Cells were held at −90 mV and stepped from −40 mV to +60 mV at 5 mV increments (10 mV increments are shown). The currents from normal and *ras*-transfected cells have a number of common characteristics. First, these currents are abolished by replacement of intracellular potassium with Cs^+, and both currents exhibit similar sensitivities to the potassium channel blocker tetraethylammonium Cl, being blocked to about 90% at 10 mM, suggesting that the currents are carried by potassium ions. Second, the voltage dependence of activation for each is similar, with significant activation occurring at steps more positive than −10 mV (Fig. 3*B*). Third, both currents undergo partial inactivation in response to test potentials greater than +20 mV. Although the outward currents in control and *ras*-transfected AtT20 cells are qualitatively similar, they differ in two impor-

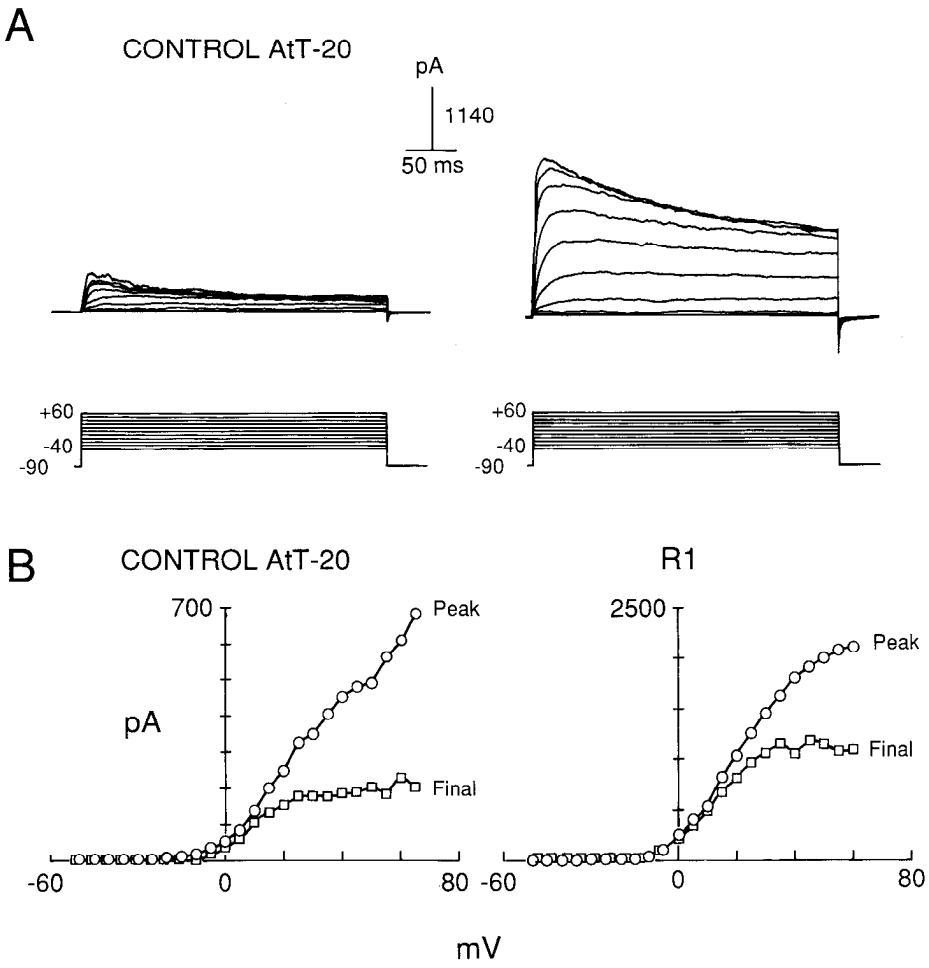

Figure 3. Voltage-dependent potassium current recorded under whole-cell voltage clamp in untransfected AtT20 cells and in EJ-*ras*–transfected clone R1 (*A*), and their corresponding *I–V* curves (*B*). The current generated in the R1 cell was about five times larger than the normal AtT20 cell current. Both cells exhibit inactivation at depolarized voltage steps. The *I–V* curves (*B*) show the peak current and current at the end of a 300 msec pulse for both cells. At depolarized steps, the differential between the peak and final currents becomes evident at voltage steps to potentials more positive than +10 mV.

tant respects. The potassium current measured in *ras*-transfected cells is approximately five times greater than that in control cells (Fig. 3*A*, Table 1). Note the differences in the current scales in the current–voltage (*I–V*) relationships (Fig. 3*B*). In part, this could reflect the increased size of the *ras*-transfected cells. However, when currents were normalized for total plasma membrane surface area, using cell capacitance as a measure of surface area, it was found that the current density is at least two times greater in the *ras*-transfected cells than in control AtT20 cells ($p < 0.0005$; Table 1). This difference was observed for both the peak current and for the partially inactivated current at the end of a 300 msec pulse to +50 mV.

Normal and *ras*-transfected cell lines also differ in the amount and rate of inactivation of their outward currents. The falling

Table 1. Differences in properties of the voltage-dependent potassium current in wild-type and *ras* tranfected AtT20 cells

Cell	Current (pA)	Current density (pA/pF) Peak	Current density (pA/pF) Final[a]	Reduction of current during 300 msec pulse (%)	Inactivation time constant (msec)	Capacitance (pF)
Wild-type AtT20	637 ± 88 ($n = 14$)	97 ± 13	55 ± 9 ($n = 14$)	44 ± 3 ($n = 14$)	92 ± 21 ($n = 16$)	6.6 ± 0.5 ($n = 14$)
Clone R1	3250 ± 379[b] ($n = 9$)	183 ± 19[b]	128 ± 16[b] ($n = 9$)	30 ± 4[c] ($n = 9$)	160 ± 17[d] ($n = 9$)	17.8 ± 2.1[b] ($n = 9$)

Cells were stepped to +50 mV from a holding potential of −90 mV. Significance was determined by Student's *t* test. Data are presented as mean ± SEM.

[a] Current at end of 300 msec pulse.

[b] $p < 0.0005$.

[c] $p < 0.005$.

[d] $p < 0.05$.

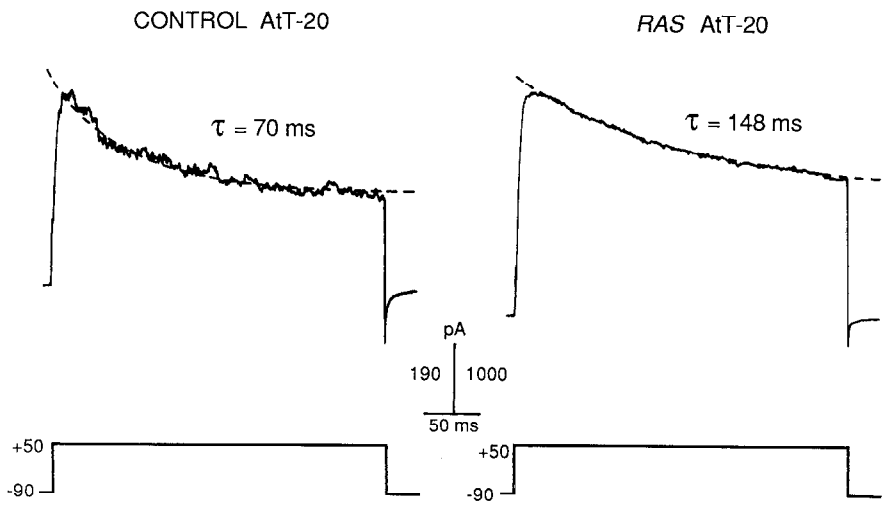

Figure 4. Potassium currents from a normal AtT20 cell (*left*) and a cell from *ras*-transfected clone R1 (*right*) fitted to curves for a single exponential (*broken line*).

phase of the outward currents could readily be fit to a single exponential curve in both control and *ras*-transfected cells. This is shown in Figure 4, where the currents evoked by stepping the membrane potential from −90 mV to +50 mV for 300 msec have been plotted at the same size for two cells. The time constant of inactivation for the *ras*-transfected cell is about twice that of the control AtT20 cell. The mean time constants for the two populations of cells are presented in Table 1. The *I–V* relationships in Figure 3*B* show both the peak current and the current recorded at the end of a 300 msec pulse. The decreased amount of inactivation of potassium current in the *ras*-transfected cells can also be seen in Figure 3. The *I–V* relationships of the two cells appear similar from −60 mV to +10 mV. Inactivation becomes apparent at steps more positive than +10 mV. The difference between the peak and final currents increases with depolarization for both cell types. The proportional decrease in current is, however, significantly greater in the control cells than in the *ras*-transfected cells ($p < 0.005$; Table 1).

ras *induces potassium channel mRNA expression*

Experiments were carried out to test whether the change in amplitude and kinetics of potassium current in the *ras*-transformed cells is associated with an alteration in the type of potassium channel genes expressed or in the level of their expression. PolyA RNA was prepared from control and *ras*-transfected AtT20 cells and subjected to Northern blot analysis using cDNA probes derived from mammalian members of each of three subfamilies of the *Shaker* family of voltage-dependent potassium channel genes. These were the clones Kv1, a member of the *Shaker* subfamily (Swanson et al., 1990); Kv4, a member of the *Shaw* subfamily (Luneau et al., 1991); and drk1, a member of the *Shab* subfamily (Frech et al., 1989). Of the probes used, a positive hybridization signal was detected only with the Kv4 cDNA probe (Fig. 5). Interestingly, only RNA from *ras*-transfected cells contained transcripts that hybridized. No corresponding signals could be detected in the same amount of RNA from normal AtT20 cells. Probes directed against the rat Kv1 gene or against drk1 revealed no hybridizing transcripts by Northern blot analysis in either the normal or *ras*-transfected AtT20 cells. Under the same conditions, a hybridization signal for Kv1 could be readily detected in RNA from rat anterior pituitary and GH3 cells (Hemmick et al., 1990; Levitan et al., 1991) and drk1 hybridizing sequences were detected in denervated skeletal muscle (J. Trimmer, personal communication).

RNA transcribed from the Kv4 gene is subject to alternative splicing and can generate two different channel species, the Kv4 channel itself and NGK2, another member of the *Shaw* subfamily of potassium channels (Yokoyama et al., 1989; Luneau et al., 1991). The sequence of NGK2 is identical to that of the Kv4 gene product over much of the coding region but differs from Kv4 at the carboxyl end of the predicted protein sequence. The sizes of the major transcripts recognized by Kv4- and NGK2-specific probes are 4.5 and 7.0 kilobases, respectively (Yokoyama et al., 1989; Luneau et al., 1991). The larger of these two is in good agreement with the size of the major species recognized by the full-length Kv4 cDNA probe in the *ras*-transfected AtT20 cells (Fig. 5). In addition, in some experiments, we detected a weaker hybridization signal at ~4.5 kilobases (not shown).

To establish further whether the Kv4 gene is expressed in *ras*-transfected cells, and to determine whether its transcript is selectively processed to yield either the Kv4 or NGK2 mRNAs, we used the technique of ribonuclease protection. For this purpose, we generated an antisense RNA probe that, at its 5′ end, was complementary to 108 nucleotides of sequence common to both Kv4 and NGK2 mRNAs. The 290 nucleotides at the 3′ end of this probe were complementary exclusively to Kv4 mRNA. A ribonuclease protection experiment should result in a 398 nucleotide protected fragment when the probe is hybridized to Kv4 mRNA and in a 108 nucleotide protected fragment when the probe is annealed to NGK2. The results of this experiment are shown in Figure 6. Both products of the Kv4 gene are expressed in rat cerebral cortex (lanes 2, 3) where the larger protected band, corresponding to Kv4 mRNA, is the predominant species. No hybridization signal was detected at either size in lanes corresponding to wild-type AtT20 cells (lanes 4, 5, 8). In RNA from the *ras*-transformed cells, however, there are protected sequences at gel mobilities expected for both Kv4 and NGK2 mRNAs (lanes 6, 7, 9). Interestingly, there is relatively more NGK2 expression in *ras*-transfected AtT20 cells. The predicted band corresponding to NGK2 mRNA appeared to be in approximately sixfold molar excess over that for Kv4 as determined by scanning densitometry and normalized for the differ-

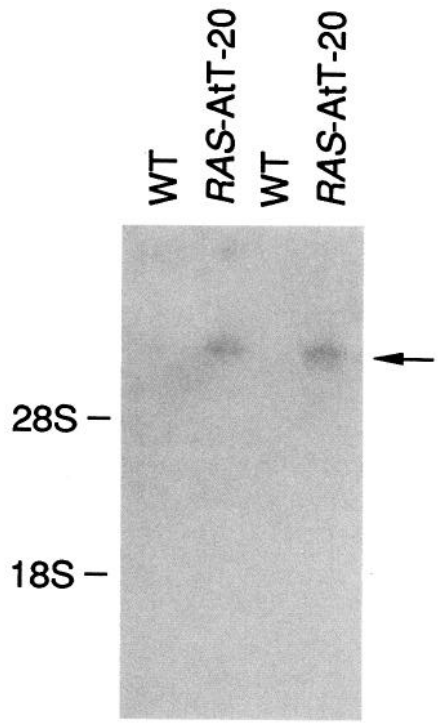

Figure 5. Induction of potassium channel mRNA recognized by Kv4 cDNA in *ras*-transfected AtT20 cells. RNA preparation and Northern blot hybridization were performed as described in Materials and Methods. *Lanes 1* and *2*, 5 μg of polyA RNA from wild-type (*WT*) AtT20 cells and clone R1, respectively; *lanes 3* and *4*, 10 μg of polyA RNA as in *lanes 1* and *2*, respectively. The mobilities of the 18S and 28S ribosomal RNA markers, 2.0 and 5.1 kilobases, respectively, are indicated on the *left* side of the figure. The *arrow* denotes the bands corresponding to the NGK2 mRNA species.

ences in the number of cytidine residues in each band. Thus, *ras* appears to induce potassium channel mRNAs corresponding to members of the *Shaw* subfamily of potassium channel genes.

Discussion

We have shown that transfection of the EJ-*ras* oncogene into AtT20 cells induces a major change in their electrical properties. *Ras* transfection causes a decrease in the width of spontaneous and evoked action potentials. This is accompanied by an increased density and decreased rate of inactivation of the voltage-dependent potassium current, and by the appearance of new mRNA species that encode voltage-dependent potassium channels.

It is likely that the induction of the Kv4 gene, brought about by *ras* transfection, results in the synthesis of new potassium channel proteins and contributes to the changes in the characteristics of the potassium currents. The kinetics of the potassium currents in *ras*-transfected cells do not exactly match those of either Kv4 or NGK2 when these are expressed in *Xenopus* oocytes. In particular, neither Kv4- nor NGK2-induced currents in oocytes undergo significant inactivation during depolarizations of 200 msec (Yokoyama et al., 1989; Luneau et al., 1991) whereas the currents in *ras*-transformed cells inactivate with a time constant of 160 msec. The protein products of potassium channel genes are, however, believed to form either homo- or hetero-oligomers with each other (Christie et al., 1990; Isacoff et al., 1990) or with smaller subunits (Rehm and Lazdunski, 1988). It is possible, therefore, that the currents in *ras*-transformed cells may result from the formation of heteromultimeric complexes of Kv4 or NGK2 with other channel subunits that coexist in both control and *ras*-transfected cells. Alternatively, Kv4 or NGK2 proteins in *ras*-transfected cells may form homomultimeric channels whose kinetics differ from the oocyte cur-

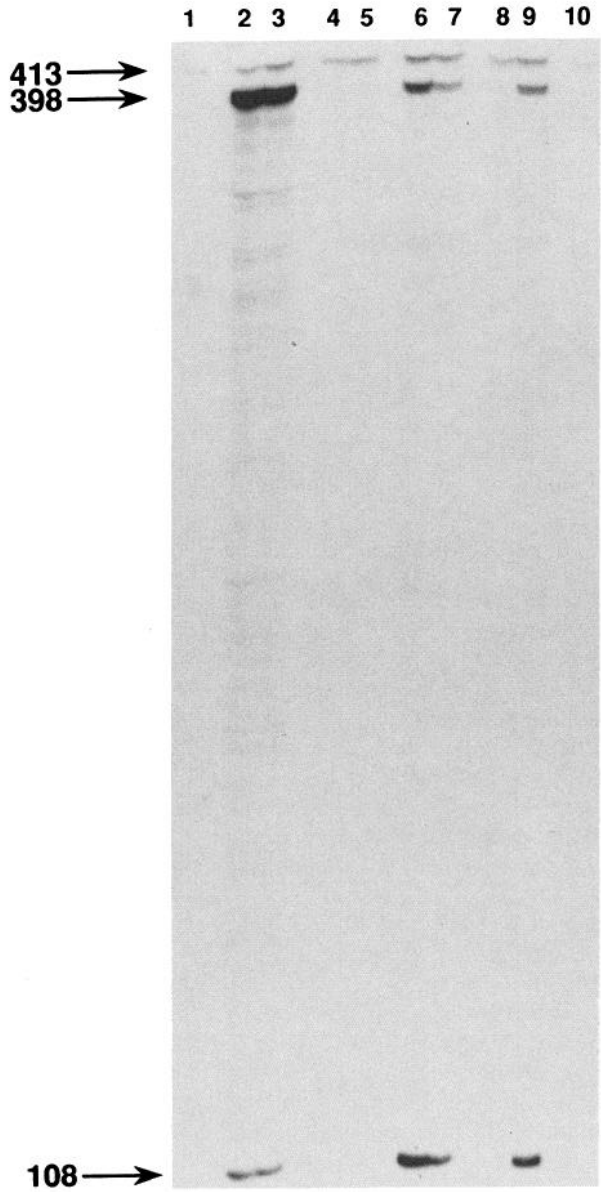

Figure 6. Ribonuclease protection assay for Kv4 and NGK2 mRNAs. *Lane 1*, 30 μg of yeast transfer RNA; *lanes 2* and *3*, 30 and 15 μg of total RNA from rat cerebral cortex, respectively; *lanes 4* and *5*, 15 and 30 μg of total RNA from normal (wild-type) AtT20 cells, respectively; *lanes 6* and *7*, 30 and 15 μg of total RNA from *ras* oncogene–transformed clone R1, respectively; *lanes 8* and *9*, 1 μg of polyA RNA from normal AtT20 cells and *ras*-transformed clone R1, respectively; *lane 10*, 500 cpm of probe. *Lanes 1–9* were digested with 6 μg/ml RNase T1 and 2 μg/ml RNase A for 1 hr at 34°C. A small amount of residual undigested full-length 413 nucleotide probe can be detected at the top of the gel. This is due to incomplete removal of the template DNA and is constant in all lanes with or without added RNA and therefore does not interfere with the interpretation of the protected bands.

rents because of their association with non-*Shaker*-like proteins found in AtT20 cells or because of posttranslational modifications that do not exist in these oocytes.

Transcription from the Kv4 gene yields two mRNAs, the Kv4 channel species and NGK2, another member of the *Shaw* subfamily of potassium channels (Luneau et al., 1991). NGK2 was first cloned from NG108-15 cells, a mouse–rat neuroblastoma–glioma hybrid cell line (Yokoyama et al., 1989). Its sequence is almost identical to that of the Kv4 cDNA sequence, isolated from rat brain mRNA, over much of the coding region, including all of the hydrophobic presumed membrane-spanning regions that are believed to constitute the voltage sensors and the ion pore itself. It diverges from Kv4, however, at the carboxyl end, which is predicted to be a cytoplasmic tail (Luneau et al., 1990). The lack of total sequence identity at the 5′ end of the cDNA is possibly due to species variation since Kv4 is a rat brain clone and NGK2 was cloned from a mouse rat neuroblastoma–glioma hybrid cell line. The results of Northern blot analysis and ribonuclease protection assays indicate that both forms exist in the *ras*-transfected AtT20 cells but that the mRNA for NGK2 is found at higher levels than that for the Kv4 channel. No trace of either form could be found in the control cells, and the identity of the potassium channel genes expressed in the untransfected cells is not yet known.

A *ras*-induced change in the amplitude and kinetics of potassium currents need not occur solely through the synthesis of a new complement of ion channel proteins. It is possible that activated *ras* proteins produce a posttranslational modification of preexisting or newly induced potassium channels in AtT20 cells, or that such channels interact directly with $p21^{ras}$ and its associated GAP. For example, putative allosteric interactions of an inwardly rectifying potassium channel with the *ras*-GAP complex occur in isolated membrane patches from guinea pig atrial muscle (Yatani et al., 1990). It is not yet known if such direct effects of the *ras* protein on the delayed-rectifier potassium channels contribute to the change in amplitude or kinetics of these currents in AtT20 cells.

The *ras* oncogene-induced transition in the shape of action potentials, which is coupled to a change in morphology to a neuronlike form, resembles the changes in action potentials that occur in many neurons during their development (Spitzer and Lamborghini, 1976; Henderson and Spitzer, 1986), and probably results from changes in more ion currents than we have described here. In a previous report, we found that control AtT20 cells express a TTX-insensitive sodium current that accounts for approximately 40% of the total voltage-dependent sodium current in these cells. After transfection with activated *ras*, however, this TTX-insensitive current is eliminated (Flamm et al., 1990). The loss of such a current also occurs during the normal development of a number of excitable cells. Preliminary evidence, using calcium-indicator dyes, also suggests that calcium currents may differ in their amplitude and pharmacology in the control and *ras*-transfected AtT20 cells (N. C. Birnberg and L. M. Hemmick, unpublished observations).

The introduction of the *ras* oncogene has been shown to alter the characteristics of ionic currents in cell lines other than AtT20 cells, such as 3T3 fibroblasts and BC3M1 myocytes (Caffrey et al., 1987; Chen et al., 1988; Rane, 1990). Our studies, in a pituitary cell line, suggest that such changes may occur through the expression of a novel set of ion channel genes. Moreover, because the Kv4 gene is normally expressed in the nervous system, our results support the hypothesis that a *ras* protein plays a role in the differentiation of cells to a neuronal-like phenotype.

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