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PRINCIPAL INVESTIGATOR: Lynne A. Fieber, Ph.D.

CONTRACTING ORGANIZATION: University of Miami
Miami, Florida 33149-1098

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INTRODUCTION

The subject of this research is a specific potassium (K) channel, the delayed rectifier K channel, which has been shown to be a key component effecting proliferation of developing Schwann cells (SC; Konishi 1990). This is a K channel that is opened in response to depolarizations of the cell membrane, which could occur in myelinating SC via excitatory discharge of the SC-ensheathed axon. In preliminary studies the delayed rectifier K channel was found to be functionally expressed in NF1 SC, but not in normal SC (Fieber 1998). The purpose of this study is to examine the inter-relationships between K channels, ras, and neurofibromin in SC proliferation. This study will aid our understand of how mutant neurofibromin contributes to the tumorigenic process. Ion channels are membrane proteins that mediate electrical communication between cells of the nervous system and are vital to nervous system function. The first year of study has suggested the role of voltage-gated K channels in NF1 SC proliferation from voltage clamp electrophysiological measurements (Fig. 1) and proliferation assays.

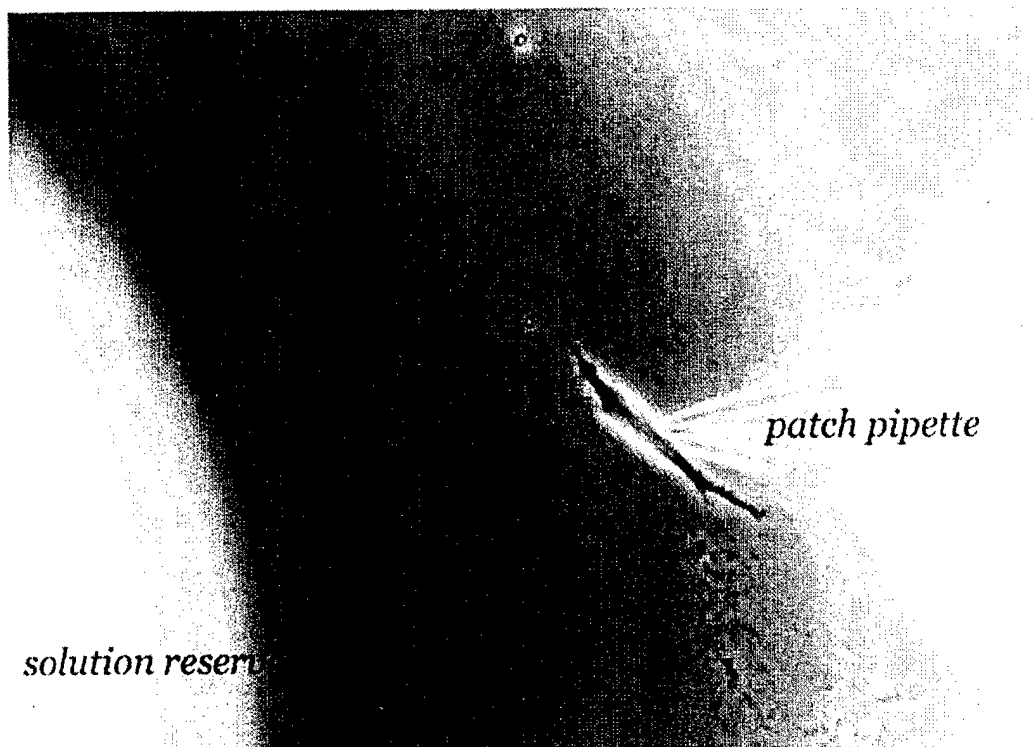


Fig. 1. A single cell voltage clamp experiment.

BODY

NARRATIVE OF TIME LINE FOR THE PAST YEAR OF THE PROJECT

The scientific goals of the first year of this study encompass Technical Objective 1 outlined in the Statement of Work. In this objective, we planned to pharmacologically block tumored K channels in primary cultures of neurofibroma- and neurofibrosarcoma-derived SC to demonstrate that block of K channels inhibits proliferation. In addition, to evaluate the specificity on inhibition of proliferation of *tumored* K channel block, we planned to pharmacologically block proliferating normal SC, which were not expected to have tumored K current.

During September 1999 – January 2000, the morphology and electrophysiological characteristics of normal SC from The Miami Project to Cure Paralysis were studied to determine the optimal method for obtaining proliferating cultures of normal SC. Normal SC are quiescent *in vitro* (Casella 1996). Experiments were performed with recombinant human heregulin, forskolin or basic fibroblastic growth factor in the culture medium to stimulate proliferation. From these experiments it was concluded that human recombinant heregulin (GGF2), as an additive to complete culture medium, gave the best results for preserving the bipolar morphology characteristic of normal SC and maintaining general cell health as assessed by gross indicators such as the degree of vacuolization in the cell cytoplasm and normal resting potentials. GGF2 was loaned to this study by Dr. Patrick Wood of The Miami Project through his collaboration with Dr. Mark Marchionni of Cambridge Neuroscience (Cambridge, MA). We have recently learned that recombinant heregulin is available from R and D Systems of Minneapolis, MN, a commercial source for the heregulin necessary to execute the experiments in which the role of K channels in proliferation of normal SC is studied.

Results of experiments with normal SC are summarized in RESULTS, together with the results of experiments on tumored SC.

Another testing effort ongoing is that of the commercially available test kits for assessing intracellular concentrations of cyclic adenosine monophosphate (cAMP) by non-radioactive methods. Many of the experimental manipulations planned on human SC in this study are likely to change the cAMP status of the cell. We anticipate that this important second messenger may link SC K channels to neurofibromin in determining certain physiological states of the cells.

We have recently decided that the kit sold by Sigma Aldrich (St. Louis, MO) may be the best for our needs. This kit is currently back-ordered with no estimated delivery date.

During this same period extensive effort was expended to find a postdoctoral fellow to assist in the project. The P. I. participated in a job workshop at the 1999 meeting of the Society for Neuroscience, including face-to-face interviews with approximately 7 interested candidates. In addition, 2 advertisements were run in the on-line job service publication of the Federation and Societies for Experimental Biology (FASEB), and two more advertisements were run in the print and on-line versions of the Neuroscience Newsletter. 3 applicants resulting from these efforts were interviewed and 2 were judged suitable for the position, but both declined the job.

After obtaining permission from the USAMRMC to broaden the search to include other suitable applicants who could be fit into the training aspect of the grant, such as graduate students, beginning graduate student Diana Gonzalez was hired to work on the project July 1, 2000.

In January 2000 a collaboration with Dr. Peggy Wallace and Dr. David Muir of the University of Florida was established, as a consequence of Dr. Wallace inviting the P. I. to speak at a Florida Chapter Meeting of the National Neurofibromatosis Foundation (NNFF; see Appendix). This collaboration led to Dr. Muir sharing frozen stocks of numerous of his and Dr. Wallace's human SC cultures (Wallace et al. 2000), including normal nerves from an NF1 patient, the subject of Technical Objective 3. These cultures are enriched for SC as determined by immunoassay for S-100. Due to the unexpected availability of these primary neurofibroma cultures and cell lines, work in Technical Objective 1 switched to study of tumored SC cultures. Dr. Muir and Dr. Wallace have dozens of NF1 cultures they have offered to the project.

Since up to this point experiments on the tumored K current had been limited to studies of the long-established cell lines ST88-14, 88-3, and 90-8 (Fieber 1998) it was critical to determine if other tumored SC cultures and cell lines such as those supplied by Dr. Muir had the same electrophysiological characteristics as ST88-14, 88-3, and 90-8. Two months were invested in recording from these tumored cell cultures and evaluating the reproducibility of results in Fieber 1998.

The P. I. attended the NNFF meeting in Aspen, Colorado, June 4-7, 2000, to present preliminary data (see Appendix). At this meeting a collaboration was established with Dr. George De Vries of Hines Veterans Hospital and Loyola University, Hines, IL who provided the excellent human neurofibrosarcoma cell line T265 (Badache and De Vries 1998; Badache et al.

1998) which has been instrumental in working out details of the proliferation inhibition assay described below.

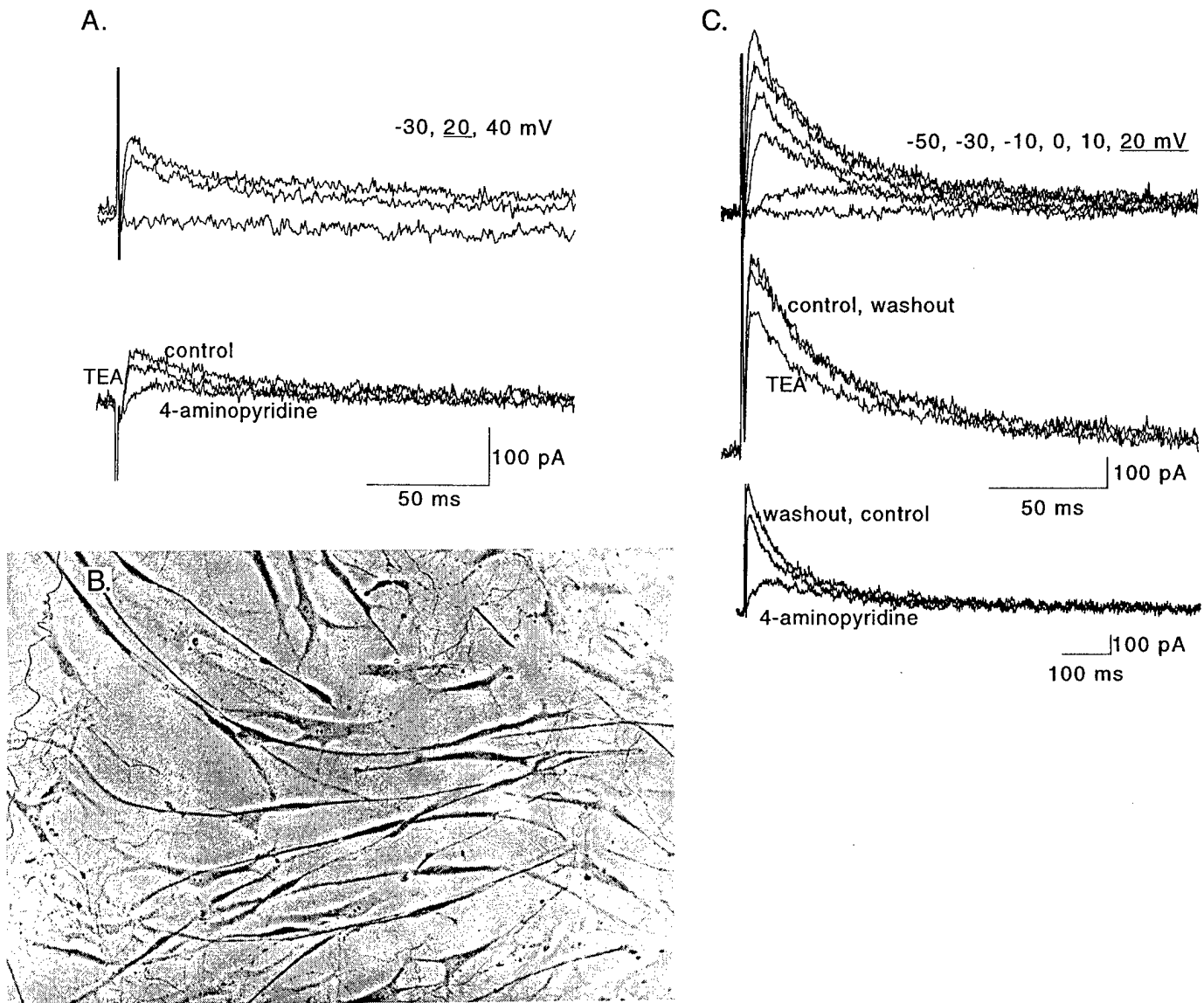


Fig. 2. K currents in normal SC.

A. K currents in normal SC plated on collagen but naïve to GGF2 or other exogenous growth factors. Family of K currents to the indicated test potentials (top) and pharmacological experiments at 20 mV (bottom) to identify the K currents as inactivating, A type K currents preferentially blocked by 4-aminopyridine. These same currents also had a small tetraethylammonium- (TEA-) sensitive component.

B. Photomicrograph corresponding to the normal cells in part A.

C. K currents in a different culture of normal SC plated on laminin many days after withdrawal of GGF2 from the culture medium. Family of K currents (top) and pharmacological experiments (middle and bottom) similar to part A.

RESULTS

Fig. 2 shows K currents of cultured normal SC treated 2 ways: 1) quiescent cultures that were not exposed to agents that stimulate proliferation (Fig. 2A and a photomicrograph Fig. 2B), or 2) cultures of proliferating SC exposed in culture to medium containing glial growth factor 2 (GGF2) soon after their dissociation from peripheral nerve, but then maintained in normal culture medium for several days before electrophysiological recordings were made (Fig. 2C). Normal culture medium consisted of Dulbecco's Modified Eagle's Medium (DMEM) plus 15% heat inactivated fetal bovine serum with 50 U/ml penicillin and 0.05 mg/ml streptomycin (all from Life Technologies, Grand Island, NY). Cultures of normal SC were enriched for SC (Casella 1996; Wallace et al. 2000). As in Fieber 1998, quiescent normal SC exhibited predominantly fast-inactivating A type K currents blocked by the specific A current blocker, 4-aminopyridine, and to a lesser extent (0-90% of control K current amplitude), by tetraethylammonium (TEA; Fig. 1A). A type K currents with similar pharmacology also were recorded from GGF2-exposed SC cultures (Fig. 2C). The latter culture was neurofibromin-positive (David Muir, personal communication).

Fig. 3 shows K currents of cultured SC from a cutaneous neurofibroma (Fig. 3A and a photomicrograph Fig. 3B) and from a neurofibrosarcoma (Fig. 3C); SC in these cultures proliferate in normal culture medium. K currents of tumored SC from the cutaneous neurofibroma were similar to normal SC K currents: A type inactivating K currents blocked by 4-aminopyridine. This culture was neurofibromin-negative (David Muir, personal communication). Experiments to evaluate the K currents of additional, non-malignant NF1 tumors, which have been obtained from Dr. Muir, are planned in year 2 of the study.

Tumored SC K currents from the T265 neurofibrosarcoma cell line were typical tumored K currents of Fieber 1998: non-inactivating and preferentially blocked by TEA (Fig. 3C). These results establish the presence of the tumored K current in malignant NF1 tumored SC. This culture was neurofibromin-negative (Badache et al. 1998).

Table 1 summarizes the K current results on normal and tumored SC.

Fig. 4 and Table 2 illustrate the results of 70 hr cell proliferation studies conducted in the neurofibrosarcoma cell line T265 using two TEA analogs to inhibit proliferation. We followed the procedure of Wilson and Chiu (1993), with optimization of drug concentration for human cell cultures. Cell proliferation was assessed by the bromodeoxyuridine (BrdU) assay kit (Zymed Laboratories, South San Francisco, CA) instead of via incorporation of H³-thymidine as

proposed because of the combination of ease of use of the BrdU assay (results are immediate after completion of the 2 hr assay) and because it measures the same parameter as the

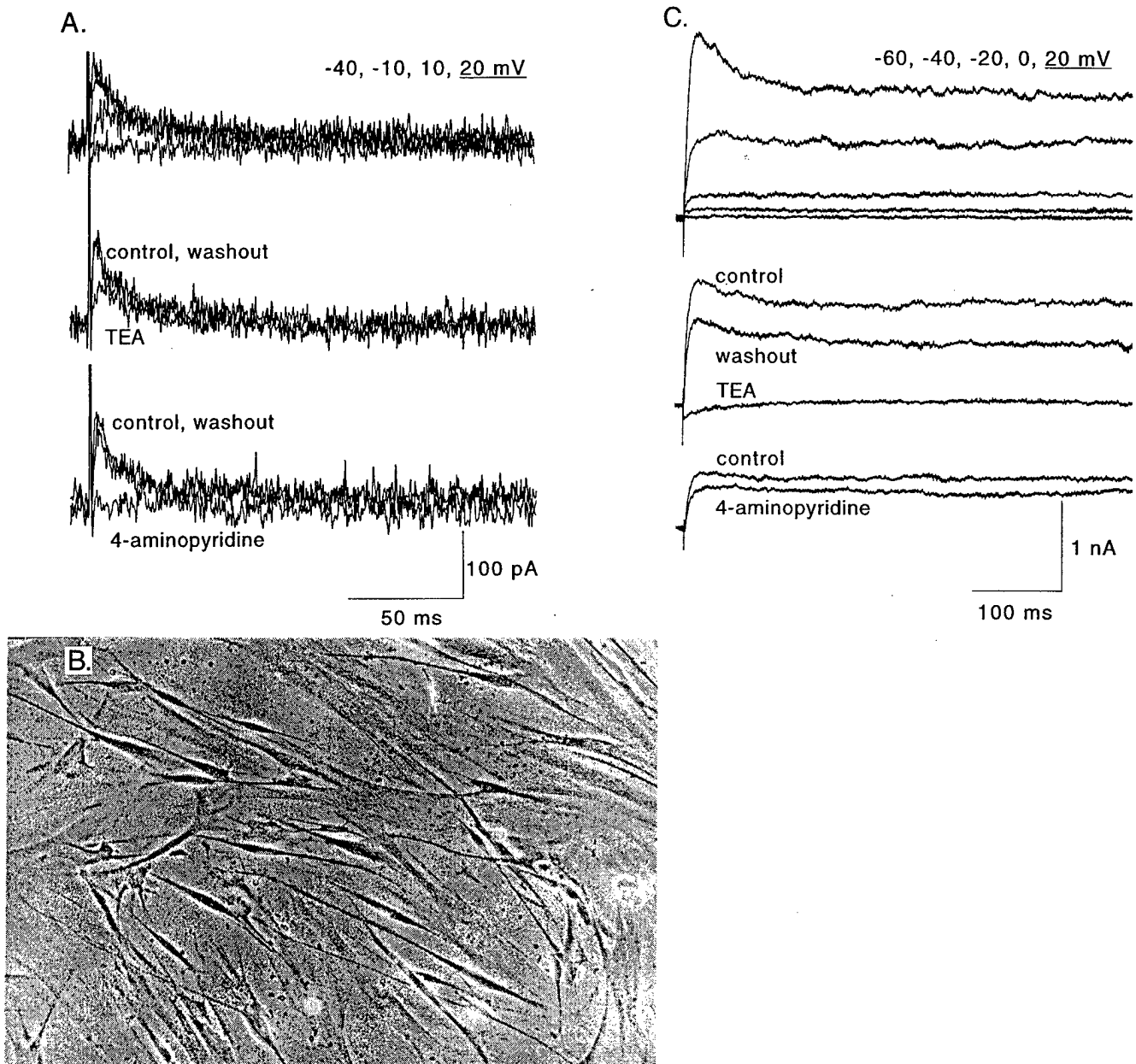


Fig. 3. K currents in SC from NF1 tumors.

A. K currents in SC from a cutaneous neurofibroma plated on laminin many days after withdrawal of GGF2 from the culture medium. Family of K currents (top) and pharmacological experiments (middle and bottom) to identify the K currents as inactivating, A type K currents preferentially blocked by 4-aminopyridine. These same currents also had a small TEA-sensitive component. B. Photomicrograph corresponding to the neurofibroma-derived SC in part A.

C. K currents in a different culture of neurofibrosarcoma SC T265. Family of K currents (top) and pharmacological experiments (middle and bottom) demonstrating the non-inactivating, delayed rectifier K current ("tumored K current") preferentially blocked by TEA.

Culture Type	Growth factor-exposed	Neurofibromin immunoassay	Outward K current	4-AP block	TEA block
Normal SC	No	Not determined	7/11 (64%)	6/7 (86%)	3/4 (75%)
Normal SC	GGF2 for several weeks after dissociation	Positive	10/13 (77%)	8/8 (100%)	7/8 (88%)
Cutaneous neurofibroma SC	No	Negative	11/13 (85%)	6/6 (100%)	1/8 (13%)
Neurofibrosarcoma SC T265	No	Negative	21/21 (100%)	15/15 (100%)	17/17 (100%)

Table 1. Summary of K current pharmacology in cultures of normal and NF1-affected SC. 4-AP: 4-aminopyridine. Note that numbers in parentheses denote the percent cells blocked by 4-AP or TEA, not the percent current per cell that was blocked. In both normal cultures and the neurofibroma the percent K current blocked by TEA was usually low at <30%, while block by 4-AP was usually high, at >70%. Conversely, in the T265 cell line, the percent K current blocked by 4-AP was usually low, at <50%, while block by TEA was always high, at >90%.

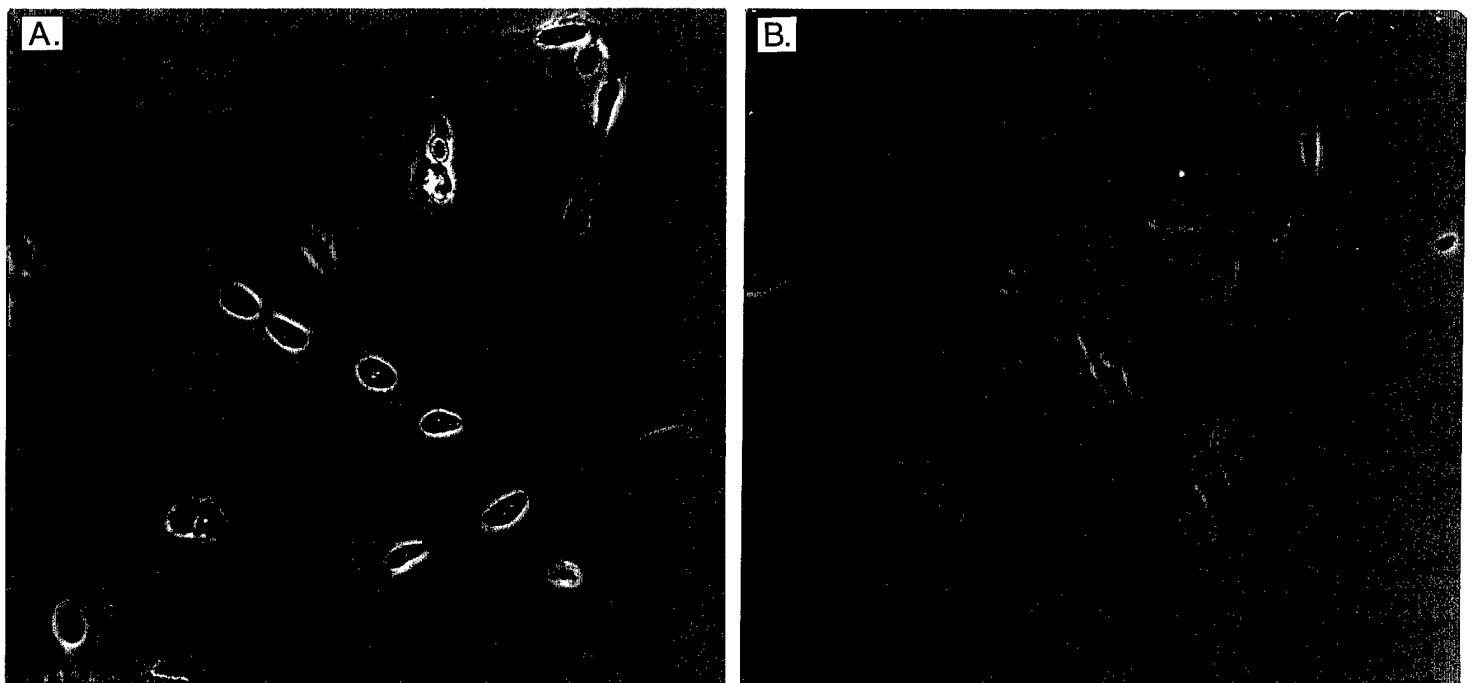


Fig. 4. BrdU assay to assess cell proliferation in the T265 neurofibrosarcoma cell line under different conditions.
 A. Proliferating control culture with cell division illustrated by the dark, BrdU-staining nuclei.
 B. Matched culture of T265 cells in 5 μ M THeA for 48 hrs, lacking BrdU-positive-staining.

H³-thymidine assay: cell division over time. In the BrdU test, BrdU-positively stained cell nuclei denote that the cell divided during the period of BrdU exposure (Fig. 4A).

Each of the two TEA analogs, tetrapentylammonium (TPeA; 50 μ M) and tetrahexylammonium (THeA; 5 μ M) completely inhibited tumored SC proliferation during 70 hrs of continuous exposure (Table 2). A second BrdU experiment demonstrated that 48 hrs' exposure to these agents resulted in a 76% reduction of proliferation in SC exposed to TPeA and a complete inhibition of proliferation in SC exposed to THeA and (data not shown).

Treatment	Number of cells	% BrdU-positive cells	Resting Potentials	K current amplitude
50 μ M TPeA for 70 hrs	27.2 \pm 3.15*	0 \pm 0*	-19.3 \pm 10.3 mV ⁺	0 \pm 0 pA (44 hrs)*/ 63.3 \pm 63.3 pA (66 hrs)**
Control (no TPeA)	74.5 \pm 3.20*	51.3 \pm 3.81*	-44.6 \pm 14.0 mV	486 \pm 75.6 pA
No BrdU control (+TPeA)	30.0 \pm 6.24	0 \pm 0	-----	-----
No primary antibody control (+TPeA)	24.0 \pm 6.24	0 \pm 0	-----	-----
5 μ M THeA for 70 hrs	27.3 \pm 4.94*	0 \pm 0*	-16.6 \pm 20.6 mV ⁺⁺	110 \pm 24.4 pA (41 hrs)*/ 30.2 \pm 92.5 pA (69 hrs)**
Control (no THeA)	45.5 \pm 0.50*	45.7 \pm 1.30*	-44.6 \pm 14.0 mV ^{**}	486 \pm 75.6 pA ^{**}
No BrdU control (+THeA)	17.7 \pm 1.15	0 \pm 0	-----	-----
No primary antibody control (+THeA)	19.0 \pm 15.6	0 \pm 0	-----	-----

Table 2. Effects of TEA analogs on cell proliferation and electrophysiological parameters. Both BrdU and electrophysiology experiments were performed on the same passage/cell plating. BrdU data represents one experiments in which the cells in 3 fields at 200x magnification were counted in each of 2 dishes in the TEA analog or its positive control, and in 3 fields of 1 dish for each of the 2 negative controls. Another experiment gave similar results, with no positive BrdU-positive cells in dishes exposed to TEA analogs (see text).

*Means \pm SE, all others are means \pm SD.

⁺ values for 44 and for 66 hrs in TPeA have been combined. TPeA was applied 20 hrs after cell plating.

⁺⁺ values for 41 and for 69 hrs in THeA have been combined. THeA was applied 20 hrs after cell plating.

^{**}the same control cells were used as controls for both TEA analog experiments spanning a time period of 61-89 hrs after plating.

[#] current was 0 pA in 3 of 4 cells measured; in the 4th cell current amplitude was 253 pA.

^{##} current was 0 pA in 3 of 5 cells measured; in the 4th and 5th cells current amplitudes were 73 and 78 pA.

Fig. 5 shows that K currents were completely blocked in cells exposed for 44 hrs to TPeA (50 μ M; Fig. 4A) and almost completely blocked in cells exposed for 41 hrs to THeA (5 μ M; Fig. 5B). Resting potentials were reduced in cultures exposed to TEA analogs, from an average of -44.6 ± 14.0 mV (mean \pm SD) in control cells (n=7) to -19.3 ± 10.3 mV in TPeA-exposed cells (n=10) and to -16.6 ± 20.6 mV in THeA-exposed cells (n=10). The reduced resting potentials observed in TPeA and THeA were significantly different from controls (t-test; p=0.002 for TPeA; p \leq 0.0001 for THeA).

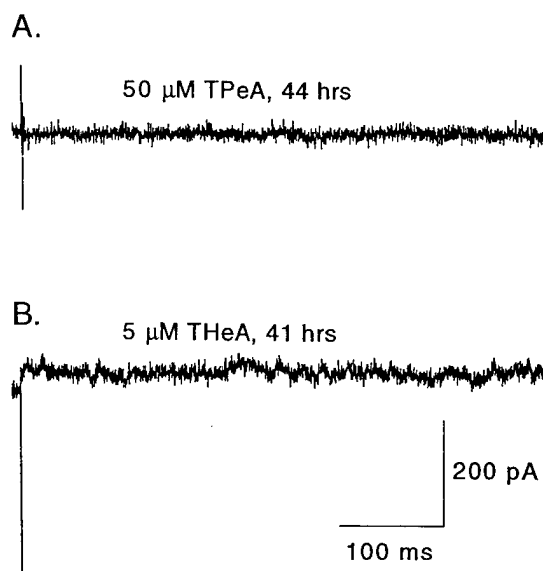


Fig. 5. K currents in T265 cells after continuous exposure to TEA analogs.
A. Outward K currents at 20 mV after 44 hrs in TPeA.
B. Outward K currents at 20 mV after 41 hrs in THeA.

K currents were blocked by exposure to TEA analogs, not down-regulated. This was demonstrated by normal amplitude K currents in tumored SC after washing off 10 μ M TPeA or 1 μ M THeA to which the cells had been exposed for 22-48 hrs. These cells were rinsed in normal saline for 20 min-1 hr before recording (data not shown). K current amplitude after TPeA washout averaged 833 ± 251 pA (n=15), while after THeA washout it was 524 ± 110 pA (mean \pm SE; n=12). K current amplitude in matched controls was 1422 ± 322 pA (n=16). These K current amplitudes were not significantly different. Resting potentials measured in TPeA-exposed cells 20 min - 1 hr after washing out the TPeA were normal, at -45 ± 18 mV (mean \pm SD; n=8) versus -49 ± 8.7 (n=3), for controls. These resting potentials were not significantly different. Resting potentials in cultures after prolonged washout of THeA have not yet been measured.

There is a possibility that inhibition of proliferation is correlated not with block of the tumored K current, specifically, but with dissipation of the normal resting potential in cells exposed to TEA analogs. We plan to test this alternate hypothesis during October, 2000, by depolarizing T265 cells 25-30 mV by addition of KCl to the normal culture medium for 48 hrs prior to the BrdU assay, a procedure that should have little effect on voltage-gated K channels. Another experiment, one that probably is without any effect on K channels, is to dissipate resting potential via inhibition of the Na⁺/K⁺ ATPase with ouabain. If depolarization of the cells results in inhibition of proliferation by these methods, then changes in resting potential may be the primary cause of inhibition of proliferation, with K channel block being just one mechanism for achieving it.

We plan to test inhibition of SC proliferation in NF1 via inhibition of SC K channels by treating cultures of other NF1 tumor cultures SC from Dr. Muir with TEA analogs. This week we are testing the specificity of TEA analog-induced inhibition of proliferation in other SC tumors. We are testing SC cultures of the damselfish model of NF1, damselfish neurofibromatosis (DNF; Schmale et al 1983) with TEA analogs.

The usefulness of technique of flow cytometry in the proliferation studies reported above has not yet become apparent. Although it is a fast and easy technique that gives a detailed view of the distribution of cells in every step of the cell cycle, since it represents a snapshot in time of a large number of cells, the contribution of cells that have already divided and are back in G₀ to the quantitation of proliferation are lost, so it is not useful for estimates of overall population increases. Total increases in the population are currently a more useful indicator for our studies. If resting potential is implicated in proliferation, studies of where in the cell cycle the arrest is occurring in response to low resting potential may be relevant. At this time, flow cytometry will be used to understand the specific part of the cell cycle that is inhibited in circumstances of block of proliferation.

KEY RESEARCH ACCOMPLISHMENTS

1. K currents in a cutaneous neurofibroma resembled those of normal SC more than those of tumored SC. This raises the possibility that neurofibroma SC ion channels are not physiologically abnormal or that the ion channel phenotypes of normal, neurofibroma-derived

and neurofibrosarcoma-derived SC represent a continuum that requires closer observation for differences between normal and neurofibroma-derived SC.

2. The neurofibrosarcoma cell line T265 had tumored K currents like those of 3 other neurofibrosarcoma-derived cell lines. T265 cells, which are easy to maintain in the lab, have proved useful in beginning experiments to understand the role of K channels in SC proliferation.

3. TEA analogs applied for 41-69 hrs' continuous exposure to T265 neurofibrosarcoma-derived SC block K channels, block proliferation, and decrease resting potentials.

REPORTABLE OUTCOMES

1. Invited talk by the P. I., January 8, 2000 at the Regional Chapter meeting of the NNFF, Orlando, FL: "A new view of the Schwann cell in understanding neurofibromas."

2. New collaboration with Dr. Peggy Wallace and Dr. David Muir of the University of Florida, whose labs have agreed to provide NF1 cell cultures for this project. This has led to the establishment of human NF1 tumored cell lines and the acquisition of primary cultures of normal human SC in the P. I.'s laboratory.

3. New collaboration with Dr. George De Vries of Hines Veterans Hospital and Loyola University, Hines, IL, whose lab provided NF1 neurofibrosarcoma cultures for this project. This has led to the establishment of the T265 cell line in the P. I.'s laboratory.

4. Poster presentation, June 2000 NNFF Annual Meeting Aspen, CO: Ionic currents in normal and neurofibromatosis type 1-affected human Schwann cells.
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5. Training of beginning graduate student Diana Gonzalez in cell culture and biochemical techniques.

CONCLUSIONS

Whereas the new neurofibrosarcoma cell line studied in the past year, T265, had currents like SC from neurofibrosarcomas studied in Fieber 1998 (ST88-14, 88-3, and 90-8), the single, non-malignant NF1 tumor studied, a cutaneous neurofibroma, had K currents more like normal SC than tumored SC. This is contrary to the initial expectation that neurofibroma-derived SC

would have a tumored SC ion channel profile. It is possible that neurofibroma-derived SC are not physiologically abnormal regarding their ion channels, but this is difficult to state on the basis of data from only one neurofibroma. It also is possible that the ion channel phenotype of normal, neurofibroma-derived and neurofibrosarcoma-derived SC represent a continuum that requires closer observation for differences between normal and neurofibroma-derived SC. Subtle differences in neurofibroma K currents compared to normal SC K currents, such as differences in current amplitude per cell or differences in the decay rate of the A type K current with time during a sustained depolarization may become apparent upon electrophysiological examination of more neurofibromas of different types such as nodular, cutaneous, or plexiform. Additional neurofibromas will be studied in the year 2; several from Dr. Muir are on hand in the lab as frozen stocks. K channel antibodies are commercially available and could be used to distinguish different K channel molecular entities in normal versus neurofibroma-derived human SC.

TEA analogs applied for 41-69 hrs' continuous exposure to T265 neurofibrosarcoma-derived SC block K channels, block proliferation, and decrease resting potentials. We are at the beginning of investigations to understand if it is K channel block or the resting potential change in SC that is inhibiting proliferation.

Dissipation of normal resting potentials when K channels are blocked probably occurs because cells are dependent on their voltage-gated K channels to maintain normal housekeeping functions of the cell such as resting potential. There is a possibility that inhibition of proliferation is correlated not with block of the tumored K current, specifically, but with dissipation of the typical resting potential of cells exposed to TEA analogs. If depolarization of human NF1 SC results in inhibition of proliferation, then changes in resting potential may be the primary cause of inhibition of proliferation, with K channel block being one mechanism for achieving this. This hypothesis was suggested by Wilson and Chiu (1993), who noted that rat quiescent cultured SC had resting potentials of -68 mV whereas during nerve degeneration this value averaged -42 mV. All cultured SC stimulated to divide with various mitogens, including those characterizing the degenerating nerve, had resting potentials in the range of -40 to -60 mV. These authors suggest that the resting potential range of -40 to -60 mV may be optimal for SC division and that any mechanism for achieving a resting potential outside this range will inhibit proliferation. This explanation suggests that NF1 tumors have characteristics in common with changes occurring in SC during development, where changes in resting potential drive the SC to differentiate and proliferate to ensheath the nerve (Konishi 1990). It also suggests that

resting potential should be a more closely monitored parameter in all electrophysiological experiments on normal and NF1-derived SC cultures.

We plan to continue to investigate the potential role in proliferation of K channel block. Is block of proliferation dependent on block of the tumored K current, or will block of the A type K current characteristic of normal SC block proliferation in normal SC stimulated to divide via heregulin? Our data suggests that TEA analogs will block some of the A type K current of normal SC or all of the tumored K current, whichever is the predominant current of heregulin-exposed normal SC. We also have on hand several neurofibromas that proliferate in normal culture medium (and can acquire more from Dr. Muir) whose K currents relative to proliferation will be studied.

SO WHAT

The link between K channels and proliferation has been suggested for SC (Konishi 1990; Wilson and Chiu 1993) and for cell types besides SC (Knutson et al 1997; Liu et al 1998), but is not clear how this link functions. NF1 tumored SC cultures, which span a continuum of non-malignant, non- or slowly-proliferating phenotypes to proliferating, malignant phenotypes, and to which normal cultured SC can be compared, represent a unique and relevant model for understanding the role of K channels in proliferation.

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APPENDIX

1. Copy of NNFF meeting announcement, Orlando, FL January 8, 2000
2. Copy of abstract for June 2000 NNFF Annual Meeting Aspen, CO
3. C. V. of the P. I.

**NATIONAL NEUROFIBROMATOSIS FOUNDATION, FLORIDA CHAPTER
REGIONAL MEETING**

**Saturday January 8, 2000
University Hall, SunTrust Bank (downtown Orlando)**

PROGRAM AGENDA

- 8:40 a.m. Registration and Refreshments
9:00 a.m. Welcome
- 9:10 a.m. "Neurological Features of NF1 in Children"
Michael Pollack, M.D., Neurologist, Nemours Children's Clinic, Orlando.
- 10:00 a.m. "Learning Disabilities"
Paula Young, Orange County Instructional Resource Teacher, Orlando.
- 11:00 a.m. Break
- 11:10 a.m. "A New View of the Schwann Cell in Understanding Neurofibromas"
Lynne Fieber, Ph.D., Research Assistant Professor, University of Miami.
- Noon Adjourn
- 1:30 p.m. Florida Chapter NNF Board Meeting

Speaker Phone numbers:

Dr. Lynne Fieber: 305-361-4906

Dr. Michael Pollack: 407-650-7130

Ms. Paula Young: 407-

Ionic currents in normal and neurofibromatosis type 1-affected human Schwann cells

Lynne A. Fieber¹, David Muir², and Margaret R. Wallace³

¹University of Miami Rosenstiel School of Marine and Atmospheric Science, Division of Marine Biology and Fisheries, 4600 Rickenbacker Cswy., Miami, FL 33149;

²Depts. of Pediatrics and Neuroscience, University of Florida, Gainesville, FL 32610;

³Dept. of Pediatrics, Division of Genetics, P. O. Box 100296, University of Florida, Gainesville, FL 32610

Comparisons were made of whole cell voltage clamp recordings from primary cultures of Schwann cells (SC) from normal nerves and from neurofibromas of patients affected by neurofibromatosis type 1 (NF1). The observation that pharmacological block of the K⁺ (K) currents of proliferating SC results in suppression of proliferation (Chiu and Wilson 1989, J. Physiol. 408:199-222) suggests a possible link between K current blockade and inhibition of proliferation of NF1 SC. This is an additional, previously unknown, pathway that may be altered in NF1. As described previously (Fieber 1998, J. Neurosci. Res. 54:495-506), the whole cell K⁺ (K) currents of normal and tumored SC could be divided into 3 types based on voltage activation range, pharmacology, and macroscopic inactivation: A type current, tetraethylammonium- (TEA-) only-sensitive current, and inward rectifier current. The most conspicuous difference between normal and tumored SC was the nature of K currents present. Normal SC K currents were inactivating, A type currents blocked by extracellular 4-aminopyridine (4-AP; 5 mM). TEA-only-sensitive currents, which were 4-AP-insensitive and non-inactivating, were common in neurofibroma-derived SC, but were not observed in normal SC. These results establish the abnormal ion channel profile of neurofibroma-derived SC. Additionally, the result that normal SC can be induced to functionally express tumored K current by cAMP analogs suggest that in humans as well as *Drosophila* (Guo, et al., 1997, Science, 276:795-798) the relationship between the protein product of the NF1 gene, neurofibromin, and K channel function is through specific second messenger pathways, which may, in turn, be regulated by neurofibromin levels. Supported by ES05705 and DAMD17-99-1-9578.

BIOGRAPHICAL SKETCH

NAME: Lynne Annette Fieber	POSITION TITLE: Research Assistant Professor
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EDUCATION :			
INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Michigan Coll. of Literature Science and Arts, Ann Arbor, MI	B. S.	1979	Zoology
Rosenstiel School of Marine and Atmospheric Science University of Miami, Miami, FL	M. S.	1982	Biological Oceanography
University of Miami School of Medicine, Department of Pharmacology, Miami, FL	Ph.D.	1989	Pharmacology
Grass Fellow, Marine Biological Lab, Woods Hole MA	Postdoc	1990	Intracellular calcium cycling
Washington University School of Medicine, Dept. of Cell Biology and Physiology, St. Louis, MO	Postdoc	1990-91	Carotid body ion channel physiology
University of Miami Rosenstiel School, Miami, FL	Postdoc	1992-95	marine animal model ion channel physiology

PROFESSIONAL EXPERIENCE:

- 1995-present **Research Assistant Professor** in Division of Marine Biology and Fisheries, University of Miami Rosenstiel School of Marine and Atmospheric Science (RSMAS). Current Research Responsibilities: P. I. on Department of Defense DAMD17-99-1-9578, Electrophysiological Changes in NF1 (9-1-99 to 8-31-03); Co-P. I. on NIH RR 10294, A National Resource for *Aplysia* (P. I.: Patrick J. Walsh; 5-1-99 to 4-30-05). Co-P. I. on NIH ES 05705 (P. I. Patrick J. Walsh; 4-1-96 to 3-31-01).
- 1994-1995 **Postdoctoral fellow** University of Miami RSMAS, supervised by Dr. Michael C. Schmale, P. I. on NIH NS 21997, Damsel fish Neurofibromatosis as a Model of Human NF. Role on project was the study of fish Schwann cell ionic currents as a physiological model of disease development.
- 1993-1994 **Postdoctoral fellow** University of Miami RSMAS, supervised by Dr. David J. Adams, NIEHS-sponsored Marine & FW Biomed. Sci. Center, ES 05705 to P. I. Daniel G. Baden. Role on project was to develop marine animals as physiological models. Studied fish Schwann cell ionic currents as a model of disease development; studied *Aplysia* bag cells as a model of hormonal modulation of membrane ionic currents.
- 1990-1992 **Postdoctoral Research Associate** in the laboratory of Dr. Edwin McCleskey, Washington University, NSF-funded research for which Dr. McCleskey was the P.I. Developed cultured carotid body cells from the rat as a model for studying hypoxia-induced changes in membrane ionic currents.
- 1990 **Grass Fellow** at the Marine Biological Laboratory, Woods Hole, MA, supervised by Dr. Ron Hoy, sponsored by the Grass Foundation. Studied cultured *Aplysia* bag cells as a model of intracellular calcium movement caused by hormones.
- 1985-1989 **Lucille P. Markey fellow** in the Department of Pharmacology, University of Miami School of Medicine. Developed cultured cardiac neurons from rat as a model of agonist-induced membrane ionic currents in the parasympathetic nervous system. Resulted in Ph.D. dissertation supervised by Dr. David Adams, P.I. on NIH HL 35422.

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POSTDOCTORAL SCHOLARS:

Co-sponsor 1995-1999: Sherry L. Purkerson, PhD.