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Tetrodotoxin-Sensitive Sodium Channels in a Continuously Cultured Cell Line Derived from the Adult Rat Cerebellum

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A method to establish continuously cultured cell lines from adult cerebellar cortex is reported. Clones (prepared by this procedure) were isolated from cerebellar established cultures at the 25th passage and after 15 months in vitro. One clone (UCHCC1) was maintained in culture and studied while the others were frozen. The cerebellar cell line UCHCC1 retained a neuronal-like morphology; the addition of dimethylsulfoxide (DMSO) to the culture medium elicited a reproducible morphological 'differentiation' event, characterized mainly by process extension. In 'differentiated' cells, veratridine significantly increased the uptake of ^{22}Na. Such enhanced uptake was blocked by tetrodotoxin (TTX) with a half-maximal inhibitory concentration of 0.9 nM. Binding of an [^{3}H]ethylenediamine derivative of TTX ([^{3}H]en-TTX) to the microsomal fraction prepared from same DMSO-treated cells, showed a single class of receptors with a maximal binding (B_{max}) of 173 fmol/mg protein and a K_d of 1.1 nM. Thyroid UCHT1 cells and 'undifferentiated' (cultured without DMSO) cerebellar cells, did not show significant effects of veratridine on ^{22}Na-uptake, or [^{3}H]en-TTX binding. The 'differentiated' nerve-like properties, induced by appropriate environmental manipulation, demonstrate the usefulness of cerebellar UCHCC1 cells as a model system for the developing central neuron. On the other hand, the novel transforming procedure opens new possibilities for obtaining permanent cell lines from other regions of the adult CNS.

INTRODUCTION

The mammalian central nervous system (CNS) is a complex unit composed of different neuronal and glial cell types, along with vascular and other mesenchymal cell elements. Hence, biochemical functions found in the whole brain cannot be ascribed with certainty to each one of its cell type components. To overcome this problem, the advantage of studying homogeneous populations of excitable cells has become evident in the last decade[4,5,22,23,25]. These clonal cell systems, derived from neuroblastoma or other transformed cells growing permanently in tissue culture, allow identification of specific cellular and biochemical events related to individual cell lineages.

However, at the present time, it has not been possible to generate immortal lines from discrete neuronal clusters of adult brain tissue. It is well known that normal postnatal neurons do not divide, and normal adult neuronal cells to not survive long in culture conditions. The most successful way of establishing brain cell lines requires the chemical induction of tumors in rat embryos followed by the derivation of cloned cell lines from these tumors[4,22,23]. This methodological strategy has the disadvantage that the origin of the clones is largely unknown. Other methods include the dissection of specific areas of murine or avian fetal or newborn tissue and subsequent culture, using either selection pressures to keep a low background of glial cells and fibroblasts[4], or virus infection to enhance neuronal proliferation respectively[18].

We have followed a novel and different approach to generate clonal cell lines from adult nervous tissue. In our laboratory we found that cells from cerebellar cortex explants, dissected out of adult thyroid tumor-bearing rats, after plating and repeated subculturing, underwent spontaneous transformation[7,8]. We have cloned these arising cultures and character-

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ized them in terms of growth properties, tumorigenicity and maintenance of neuronal markers.

The purpose of the present work is to characterize one of our cerebellar clones by light and electron microscopy, and to study the presence of voltage-dependent sodium channels both in cells growing in complete medium ('undifferentiated state') and after the addition of dimethylsulfoxide to induce morphological 'differentiation'. This search for excitable cell traits in our line was undertaken mainly to settle its potential use as a neurobiological model, and to eventually apply an identical neoplastic induction technique to prepare clonal lines from other adult encephalic regions of the rat.

MATERIALS AND METHODS

Animals and cell lines

A clonal thyroid cell line (UCHT1) obtained from tumors developed from iodine defficient goiter implants (second generation) in Fisher 344 mate strain rats, was used to induce subcutaneous tumors. Production of tumors and inoculation procedures of UCHT1 cells were reported elsewhere[9].

Morphologic and biochemical properties of UCHT1 cells

UCHT1 thyroid cells are epithelial small cells, which grow forming a follicle-like arrangement over culture surfaces. Periodic Acid-Schiff reagent showed a cytoplasmatic glycoprotein material resistant to amylase, and these cells produce thyroglobulin (a specific thyroid protein) and thyroid hormones[9]. All these cellular properties are not shared by UCHCC1 cerebellar cells (see below), thus favoring the notion that they are in fact arising from CNS tissue and certainly are not thyroid cells disseminated from the tumor to the cerebellum by hematogenous metastatic spread.

Thyroid tumor induction and establishment of cerebellar cultures

Ten Fisher, 6-month-old female rats were injected with 10^6 UCHT1 cells subcutaneously in one thigh of the animal. After 3 months animals have developed a tumor of 15–20 g, producing an evident emaciation state. At this stage they were anesthesized by pentobarbital and craniotomized with the aid of a hand-manipulated dental drill. Portions of cerebellar cortex were gently and rapidly dissected and placed on sterile watch glasses containing a mixture 1:1 of Eagles modified Dulbecco's and Ham's F12 media (Grand Island Biol. Co., NY, U.S.A.) without serum. Animals were killed and necropsy showed that the animals were free of metastasis in walls and content of abdominal, thoracic and cranial cavities. Ten Fisher 6-month-old female rats, used as controls, were submitted to the same procedure.

Cerebellar explants of approximately 1 mm^3 were prepared, placed in glass petri dishes and allowed to attach and grow in the same medium mixture containing 15% bovine, 2.5% fetal bovine serum, 0.015 M Hepes (N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid) buffer, pH 7.2, plus 50 mg/l streptomycin sulphate, 100 mg/l sodium-penicillin-G and sufficient glucose for a total amount of 6 g/l. Cultured explants were incubated at 36 °C, 100% humidity in an incubator with a controlled 5% CO_2–95% air atmosphere. Medium was renewed at 3-day intervals. Crowded cultures were split by trypsinization (0.19% trypsin in 137 mM NaCl, 5.4 mM KCl, 5.5 mM glucose, 5.9 mM sucrose and 0.17 mM Na_2HPO_4, 0.22 mM KH_2PO_4 buffer, pH 7.2).

Cell cultures

The UCHCC1 cell line was routinely grown in 10 cm diameter glass petri dishes in the nutrient mixture and incubation conditions already described.

For ion flux assays, cells harvested by trypsinization were plated at 50–75,000 cells per 35 mm Falcon plastic tissue culture dish and the cultures were allowed to reach confluent monolayers. The cells grew tightly attached to the bottom dishes and after extensive washing with serum-free medium, no detached cells were observed under phase-contrast microscopy. These cultures contained $1–2 \times 10^6$ cells each and were used 4–6 days after plating.

For [^{3}H]en-TTX binding assays, cells were grown in 150 mm glass dishes for one week. To ensure a clear 'differentiation' effect, preconfluent cultures were incubated in complete growth medium plus 2% dimethylsulfoxide for an additional 10 day period (see Fig. 1A and B). These cultures were used in the same manner as above for ion fluxes and [^{3}H]en-TTX binding measurements.

Sodium flux assays

Cells were assayed for veratridine-dependent sodium uptake according to the method of Caterall and Nirenberg[5] and Yannick et al.[25], with some modifications.

Cells, non-differentiated or differentiated with DMSO, were preincubated at 37 °C in 3.5 cm diameter plastic dishes for 5 min in a shallow water bath, in 1 ml of a solution that contained 140 mM choline chloride, 5.4 mM KCl, 0.8 mM $MgSO_4$, 1.8 mM $CaCl_2$, 5 mM glucose, 1.5 Hepes–Tris buffer, pH 7.4, plus 0.15 mM veratridine and 10^{-6} M tetrodotoxin (final concentration) when indicated. The medium was then removed and replaced with assay medium containing 5 mM ouabain (to inhibit the Na^+,K^+–ATPase and allow the passive influx of Na^+ to be measured in the absence of the competing process of active Na^+ extrusion), plus 140 mM NaCl, 5.4 mM KCl, 0.8 mM $MgSO_4$, 1.8 mM $CaCl_2$, 5.0 mM glucose, 1.5 mM Hepes buffer, pH 7.4, and the effectors veratridine, and TTX when appropriate in the same final concentration as above. $^{22}NaCl$, carrier-free (New England Nuclear), 1 μCi/ml was added last to the assay. The incubation was resumed for 10 min at 37 °C, or for a variable time in the case of the time-course study. At the end of the incubation, the assay medium was aspirated, and the dish washed rapidly 3 times with wash medium (163 mM choline chloride, 0.8 mM $MgSO_4$, 1.8 mM $CaCl_2$, 5.0 mM glucose, 5 mM Hepes, pH 7.4, without toxins or other effectors)[6].

The cell monolayer was dissolved with 2 ml of 1 N NaOH, and the digest counted in a gamma-counter. Protein determinations were done according to Hartree[11] using bovine serum albumin as standard.

For further characterization of the nature of the observed Na^+ influx, cells were also assayed in the presence of variable concentrations of TTX. Na^+ uptake measurements were carried out in duplicate dishes, and flux rates were normalized for the amount of cell protein in the cultures.

Binding assays with [^{3}H]ethylenediamine-tetrodotoxin ([^{3}H]en-TTX)

[^{3}H]en-TTX derivative II, (spec. act. 26 Ci/mmol) synthetized and purified according to Chicheportiche et al.[10] and Jaimovich et al.[12], was a kind gift of Professor M. Lazdunski, University of Nice, France.

Binding experiments were performed with crude UCHCC1 cell microsomes according to the method of Yannick et al.[25].

Cells, differentiated with DMSO or undifferentiated, as well as thyroid UCHT1 cells (used as control), were detached using a rubber policeman in ice-cold buffer (20 mM in Tris/HCl, 0.25 M sucrose, 1 mM EDTA, pH 7.4), centrifuged for 5 min at 1000 *g* and resuspended in the same buffer. Homogenization was performed in a Potter–Elvejhem apparatus at 4 °C (10 strokes, 1000 rev/min). Cell homogenates were centrifuged for 10 min at 1000 *g*; the pellet was discarded and the supernatant was centrifuged again for 10 min at 5000 *g*. The second supernatant was sedimented for 30 min at 100,000 *g* and the microsomal pellet thus obtained was resuspended in the homogenization buffer. Microsomal (0.1–0.5 mg of protein/ml) were incubated at 20 °C in a solution containing 20 mM Tris, HCl buffer, pH 7.4, 115 mM choline chloride, 2.5 mM potassium chloride and 1.8 mM calcium chloride, which was supplemented with varying concentration of [^{3}H]en-TTX. After 30 min of incubation, two aliquots of 0.2 ml each were filtered within 10 s through prewetted GF/B glass fiber filters (Whatman) under reduced pressure. The filters were rinsed twice with 2.5 ml of ice-cold 120 mM clorine chloride, 20 mM Tris, HCl buffer, pH 7.4. Filters were dried and their bound radioactivity was measured in a liquid scintillation counter. Samples (0.05 ml) of the incubation medium were taken for measurement of the total concentration of [^{3}H]en-TTX. The concentration of free ligand was calculated by subtracting the amount bound from the total, for each experimental point. Specific binding of [^{3}H]en-TTX was defined as the difference between the bound radioactivity measured in the absence and in the presence of a large excess of unlabeled tetrodotoxin (10 μM).

Morphological studies

Electron microscopy. Attached cells were used for transmission (T.E.M.) and scanning (S.E.M.) electron microscopy.

For T.E.M., monolayers were washed with serum-free growth medium and cells were fixed for 3 h in situ with 3% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.4, at room temperature. Cells were washed with cacodylate buffer for 30 min, post-

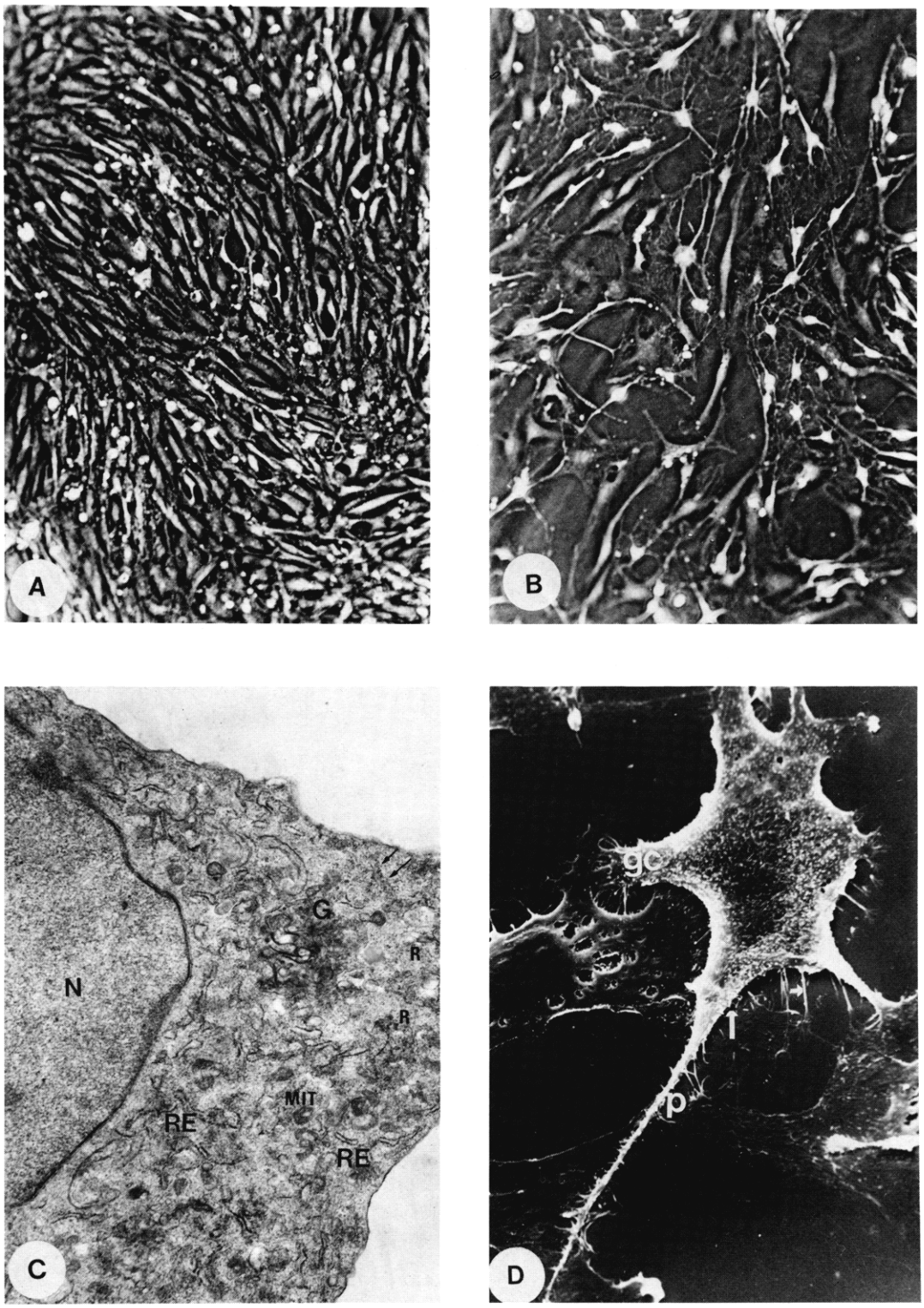

Fig. 1. 10^5 cells were seeded in plastic Petri dishes and allowed to attach overnight. After 24 h the medium was replaced by fresh medium (A, control) or fresh medium with 2% DMSO (B). In both control and DMSO-treated cultures, medium was replaced at 3-day intervals with identical media as indicated in A and B. Phase-contrast photomicrographs of living cultures taken at 10th day (× 89). C:

fixed in 1% osmium tetroxide for 1 h, washed in distilled water for 15 min and stained with 2% aqueous uranyl acetate for 1 h. After this procedure cultures were dehydrated in graded ethanol series and embedded in Epon.

After hardening of the Epon, the culture plate embeddings were examined under a light microscope and selected areas were marked, cut out, glued to specimen blocks and cut parallel to the growth surface with a LKB ultramicrotome. Sections were examined after uranyl acetate and lead citrate staining in a Philips 200 D 910 transmission electron microscope.

For S.E.M., monolayers attached to cover slips were fixed for 3 h in the same fixative as above, washed with distilled water and dehydrated with increasing concentrations of acetone up to 100%. The cells were placed in a critical point drying equipment, where acetone is replaced by CO_2 (Sorvall Critical Point System). Cultures were then coated at high vacuum (Polaron E 5000) with a layer of gold, and examined in a Siemens autoscan electron microscope.

RESULTS

Cerebellar primary cultures from rats bearing UCHT1 neoplasm

Approximately 30% of primary cerebellar explants (observed by phase-contrast microscopy) attached quickly, initiated an outgrowth in 1 or 2 days and attained confluency in 20–30 days. Part of unattached explants at the second or third week of culture, disaggregated by gently pipetting, and plated again in other dishes, formed monolayers.

In subsequent subcultures a gradual development of all cell components took place towards higher rate of division, loss of anchorage dependence, irregular growth and alteration of cell morphology. For subculturing, the established cultures were harvested by trypsinization in the above-mentioned solution (see Materials and Methods) and diluted to 1:2 or 1:20 depending on their growing characteristics. Cultures were diluted by a factor of 2:4 during the initial 4–6 passages and 5:10 during later passages. Similar findings were observed in all the 10 animal groups bearing the thyroblastoma. Cerebellar explants from normal rats attached scarcely, showed poor cell outgrowth and never achieved confluency. Passages did not proliferate and later declined and died. A preliminary account[8] of the entire procedure has already been communicated.

After being subcultured for 15 months, at the 25th passage, one of the established cerebellar strains (taken at random) was cloned by the single plating technique of Puck and Marcus[20]. Several clones were isolated; one of them was kept in culture and studied while the others were frozen.

Growth characteristics of UCHCC1 cells

The UCHCC1 clone has a doubling time of about 20 h, a plating efficiency of 20% and a saturation density of about 300,000 cells/cm^2. Cultures are mycoplasma free as determined by Bisbenzimide Hoechst Stain 33258, electron microscopy and bacteriological procedures. Morphological aspects and biochemical properties are maintained after several freezing and thawing priods. This line is tumorigenic; 10^6 cells injected subcutaneously produce tumors (10–20 g average weight) in 3-month-old adult isogenic rats. Furthermore, by peritoneal injection it is possible to develop large tumoral masses and ascites.

A confluent culture of UCHCC1 living cells, as seen by phase-contrast microscopy (Fig. 1A), shows that the cells form a monolayer tightly attached to the bottom of the glass dish, displaying roughly an epithelial fibroblastoid appearance. Although the monolayers have a rather uniform aspect, it is possible to distinguish, especially in sparse cultures, two subtypes: a few polygonal cells with radially arranged short processes, and a greater number of bipolar spindle-shaped cells, that also present short proces-

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low magnification transmission electron micrograph of a UCHCC1 cell cultivated in growth medium (sparse culture) showing a clear nucleus with regular boundaries (N). Cytoplasm is rich in rough endoplasmic reticulum (RE), mitochondria (MIT), and free ribosomes (R), some grouped in rosettes (arrows). Also there is a Golgi zone (G) (× 8550). D: low magnification scanning electromicrograph of a UCHCC1 cell cultivated in growth medium (sparse culture), showing irregular and polygonal soma; process (p); microvilli (long arrow); filopodias (short arrow) and growth cone-like zone which displays filopodias (gc) (× 1275).

ses. The addition of 2% DMSO rapidly stops cell division and after 10 days of incubation with it (Fig. 1B), the cell processes elongate markedly and new ones appear. These processes develop principally by extension, associated with ruffling activity at the tip (growth cone-like structures).

Ultrastructure

In non-differentiated cultures, cellular organization is dominated by a well-developed and diffuse system of rough endoplasmic reticulum. Perikarya also contain a random distribution of mitochondria and free ribosomes (Fig. 1C). Most UCHCC1 cells were mononucleated, showing clear nuclei with regular boundaries. The cytoplasm also contained Golgi complexes, clear vesicles and a tubule filamentous apparatus.

In scanning electron microscopy, cerebellar cells looked relatively flat with microvilli covering the surface of most of them. They varied in shape from flattened and about 30–60 μm in diameter, to polygonal- or spindle-shaped cells. Some cells displayed growth cone-like zones with filopodias (Fig. 1D).

Sodium fluxes

The time-course of veratridine-dependent $^{22}Na^+$-uptake studied in UCHCC1 cells, 'differentiated' for 10 days in 2% DMSO, is shown in Fig. 2A. As in other excitable cells[4,5,15,24], veratridine increased the rate of $^{22}Na^+$ entry into the cell, and such increase was blocked by 1 μM TTX.

A dose–response curve for the effect of TTX (Fig. 2B) indicated that half-maximal inhibition of veratridine-stimulated sodium influx occurred at 0.9×10^{-9} M TTX.

Binding of [^{3}H]ethylenediamine-tetrodotoxin ([^{3}H]en-TTX) to cell membrane

The binding of [^{3}H]en-TTX to a microsomal membrane fraction obtained from UCHCC1 cells after 10 days of DMSO treatment, showed a specific and saturable component (Fig. 3A). Non-specific binding, measured in the presence of 1 μM cold TTX, was less than 50% of total binding at 10^{-9} M [^{3}H]en-TTX. Scatchard analysis (Fig. 3B) showed that a single, high-affinity saturable binding component was present, and a dissociation constant of 1.1 nM, and a

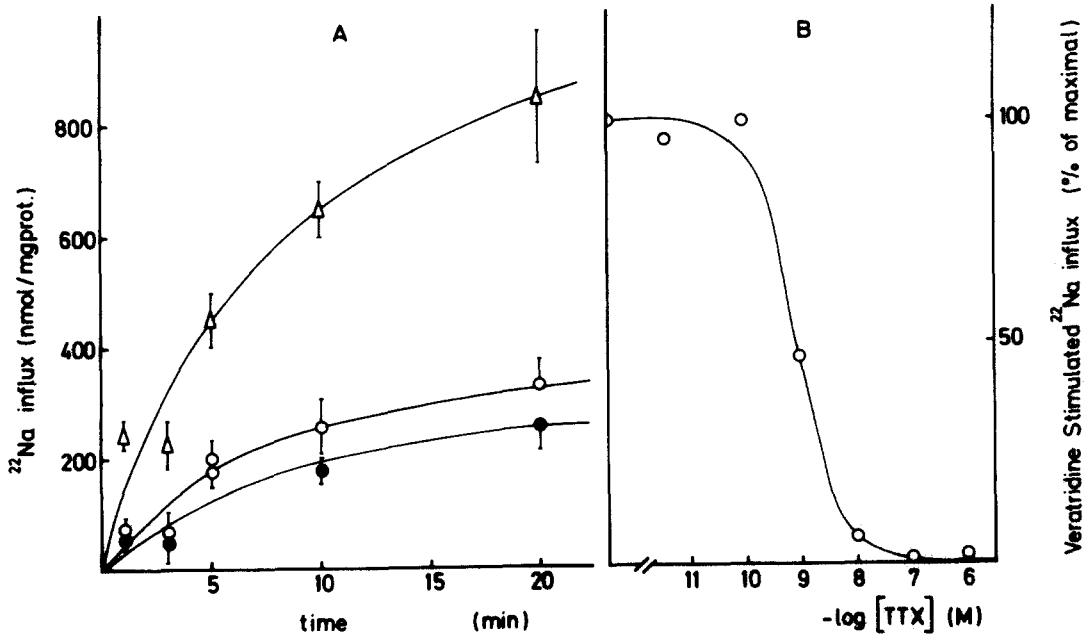

Fig. 2. A: time-course of $^{22}Na^+$ uptake for UCHCC1 cells precultured for 10 days in growth medium with 2% DMSO. $^{22}Na^+$-uptake was measured at stated times in duplicate, as described in Materials and Methods. Labeled assay medium contained additionally: ●, 5 mM ouabain; △, 5 mM ouabain plus 150 μM veratridine; ○, 5 mM ouabain, 150 μM veratridine and 1 μM tetrodotoxin. B: inhibition by tetrodotoxin of $^{22}Na^+$ uptake stimulated by veratridine in DMSO-differentiated UCHCC1 cells. Influx assays were performed as described in Materials and Methods. $^{22}Na^+$ uptake stimulated by 150 μM veratridine (ordinate) was measured in the presence of increasing concentrations of tetrodotoxin (abscissa).

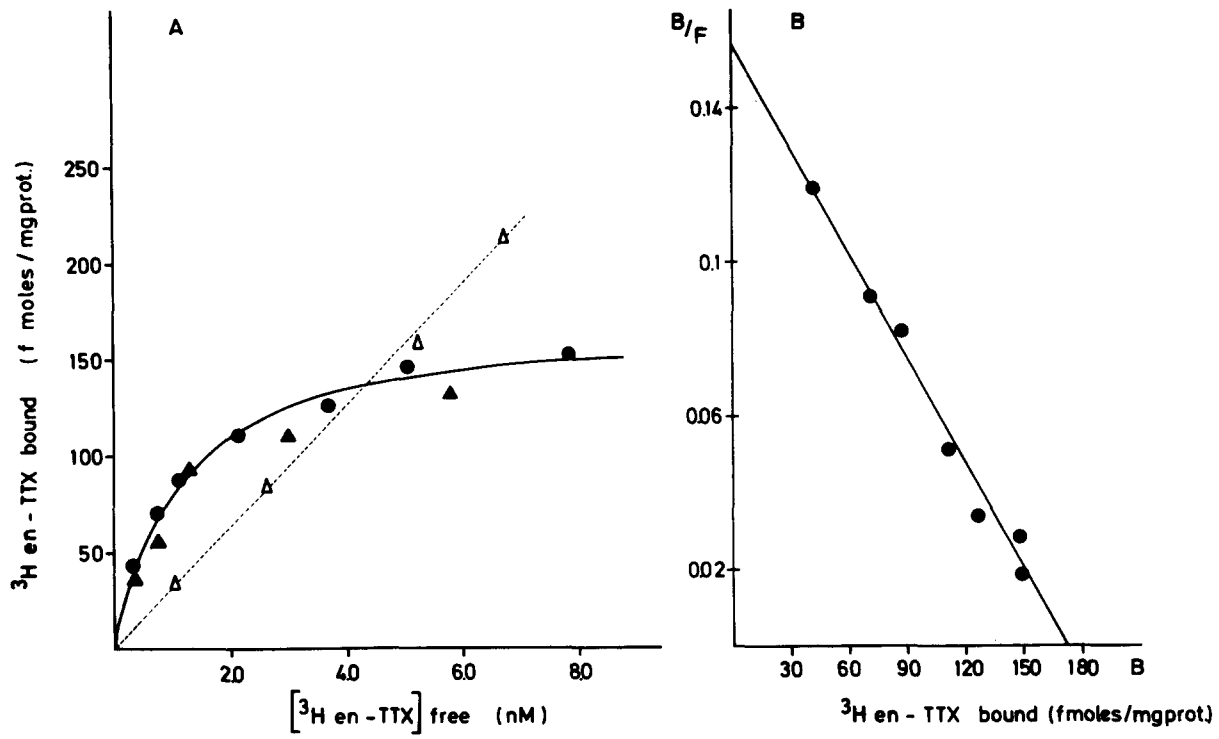

Fig. 3. Binding of [^{3}H]ethylenediamine-tetrodotoxin ([^{3}H]en-TTX) to crude microsomes from UCHCC1 cell cultures treated with DMSO. Preconfluent cultures in 150 mm glass dishes were treated with 2% DMSO for 10 days and microsomal fractions were obtained and binding measured as described under methods. A: specific binding for two independent experiments (●,▲) was obtained by subtracting the linear component obtained in the presence of 1 μM cold TTX (△) from total binding of different [^{3}H]en-TTX concentrations. B: Scatchard plot for [^{3}H]en-TTX binding to one of the experiments shown in A.

maximal binding capacity of 171 fmol/mg protein, were estimated.

A significant veratridine-stimulated, TTX-sensitive sodium influx was present only in differentiated UCHCC1 cells, not in undifferentiated ones, or in cultured thyroid cells (Table I). Something similar occurred with the TTX receptor: very little [^{3}H]en-TTX binding was seen in the thyroid cell line, even after DMSO-treatment, or in 'undifferentiated' UCHCC1 cells, but it was considerably increased after DMSO-induced 'differentiation' of the latter cell type.

DISCUSSION

A new technique to develop cell lines from primary cultures of adult rat cerebellum

Most investigators trying to establish permanently dividing cell lines from brain tissue, have accepted the currently neurobiological paradigm which states

TABLE I

Effect of DMSO treatment on $^{22}Na^+$ uptake and [^{3}H]en-TTX binding in different cell lines

Replicates of cerebellar UCHCC1 cells cultured without DMSO and UCHCC1 and thyroid UCHT1 cultured for 10 days in the presence of 2% DMSO were assayed for $^{22}Na^+$ uptake and [^{3}H]en-TTX binding (see Materials and Methods).

Cell type and culture conditions	*Veratridine-stimulated TTX-inhibited $^{22}Na^+$ uptake (nmol/min/mg protein)*	*Specific [^{3}H]en-TTX binding (fmol/mg protein)*
UCHCC1 cells	5 ± 5	22
UCHCC1 cells DMSO-treated	90 ± 18	173
Thyroid UCHT1 cells DMSO-treated	10 ± 12	< 10

that neurons from adult normal CNS tissue do not divide spontaneously, neither in vitro, nor in vivo. Therefore, in vitro attempts have been made towards the induction of neuronal division by chemical, viral or spontaneous transformation of already proliferating cells, e.g. fetal[22] or newborn[4] rat brains; otherwise CNS explants from normal adult animals degenerate and do not survive long cultivation periods. The above-mentioned paradigm applied to the in vivo situation has been challenged especially by the recent studies performed with forebrain neuronal clusters in adult canaries[17]. The results suggest that a constant turnover of neurons of two telencephalic song control nuclei takes place, in which sex hormones appear to be crucial in the recruitment of new neurons during the adulthood of the bird.

However, the paradigm still valid for CNS cultures derived from adult normal animals, seems not to operate in our primary cultures obtained from adult neoplastic animals. A consistent outgrowth is rapidly observed in cerebellar explants, a sustained proliferation is initiated after the first passage which allows clonal analysis, and the cell lineages which arose, inoculated back into isogenic animals, produced tumors. We can not interpret our results in terms of metastatic invasion from the inducer thyroid tumor to the host CNS, since no evidence of metastasis was ever detected in animal necropsies, and morphologically and functionally UCHCC1 cerebellar cells are completely different from UCHT1 thyroid cells.

The explanation for this paradoxical transformation of cultured explants dissected out an apparently normal organ derived from dying cachectic animals remains to be studied. The ability of tumors to enhance DNA synthesis on certain adult host tissues in vivo[16] is well known, and there is one report of spontaneous transformation in human skin-cultured fibroblasts obtained from neoplastic patients[2]. Usually a common cause suggested for the majority of cell transformations is an oncogenic virus. Nevertheless, transcriptase assays done in conditioned media and cell extracts of UCHCC1 and UCHT1 cells were negative (unpublished results). Also, virus-like particles were absent in electron micrographs taken in the same cells. Moreover, UCHT1 cells arose from a functional thyroid tumor experimentally induced by iodine defficiency in the same Fisher 344 rats[9]. All these collected data argue against the notion that oncegenic virus is the etiologic factor for this transformation.

Neuronal phenotype assignment to UCHCC1 cells

In order to determine whether the cell lines have physiological properties characteristic of neural tissue, the presence of voltage-dependent sodium channels was evaluated in UCHCC1 cultures growing in complete medium and in the same medium plus DMSO. DMSO is a chemical modulator which has been reported to produce process formation, together with electrical and biochemical maturation in mouse[13] and human neuroblastomas[14]. Furthermore, there is a good correlation between the ability of a cell to generate an action potential and both the rate of veratridine-stimulated $^{22}Na^{+}$-uptake[1,4,5,24] and the presence of membrane TTX receptors[15,25]. Thus, to a first approximation, these assays offer a way of screening cells for their excitable nature.

The experiments described in this paper show that when UCHCC1 preconfluent cells are treated with DMSO, differentiated cells exhibiting neuronal characteristics become the predominant type of the cell population; proliferating, undifferentiated cells are no longer seen in these cultures. Simultaneously, DMSO-differentiated UCHCC1 cerebellar cells display a veratridine-stimulated, TTX-inhibitable sodium influx, characteristic of excitable tissues[1,4,5,24]. Half-maximal inhibition (HMI) by TTX is obtained at 0.9 nM which reveals a high-affinity site, presumably due to voltage-dependent sodium channels, for this toxin. Similar HMI values have been obtained electrophysiologically on peripheral axons[1] and on mouse neuroblastoma[5]. Undifferentiated UCHCC1 cells and non-excitable thyroid cells (which apparently are responsible for the transformation of cerebellar cultures) did not show any detectable effect of neurotoxins on sodium influx, even after DMSO treatment.

[^{3}H]en-TTX binding to differentiated UCHCC1 cells showed a single class of receptors, with a dissociation constant of 1.1 nM which corresponds well with the HMI values obtained for sodium flux assays in the same cells. Both the K_d and the maximal binding capacity in this system are similar to those described for NIE-115 neuroblastoma cells[15,25].

Although some apparently non-excitable cells have been shown to have sodium channels, pharma-

cological differences between these channels and those found in NIE-115 neuroblastoma and other excitable cells exist. Channels in fibroblasts[19] and glial cells[21] ('silent channels') become apparent only after combined treatment with veratridine and a polypeptide toxin (scorpion or sea anemona toxins) but not with veratridine alone. On the other hand, these 'silent channels' have lower affinity for TTX than neuroblastoma[25] or UCHCC1 cells which can be considered excitable cells*.

It seems clear that the appearance of veratridine-stimulated sodium channels and of TTX receptors accompanies the morphological changes induced by DMSO. These results suggest that these excitability properties might have arisen by a differentiation process, since they were not observed in preconfluent or exponential cultures of UCHCC1 cells growing in DMSO-free medium. It is also evident that not all cells in culture were able to undergo such a differentiation process, since it was not seen in UCHT1 cells (Table I) nor in other cell lines from various origins (data not shown).

Our findings further show the potential of our methodological procedure for preparing a clonal system from a variety of discrete regions of the adult CNS. Particularly striking is the ability of this permanent line to elaborate the complex apparatus necessary for the expression of electrical excitability. Apparently, the neoplastic nature of UCHCC1 cells does not interfere with the possibility of evoking differentiated properties. Hence it should be feasible to derive other clonal systems from different CNS segments.

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* The term 'excitable cells' may include in the future some glial cells, since it has been recently postulated that astrocytes have TTX-sensitive sodium currents[3].

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