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Electrophysiological Properties of Ethylnitrosourea-induced, Neoplastic Neurogenic Rat Cell Lines Cultured *in Vitro* and *in Vivo*[1]

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SUMMARY

A comparative analysis was performed on the electrophysiological properties of 11 neoplastic neurogenic cell culture lines and five other cell lines of different origin (HV1C, rat bile duct carcinoma; BICR/M1R_K, rat mammary tumor; HeLa, human cervix carcinoma; 3T3, mouse embryo; REe, rat embryo). Neurogenic lines were derived either from *N*-ethyl-*N*-nitrosourea-induced neoplasms of the nervous system or from cultured fetal rat brain cells that had undergone neoplastic transformation *in vitro* after exposure to *N*-ethyl-*N*-nitrosourea *in vivo*.

Electrical membrane excitability was lacking in all neurogenic cells analyzed. Their membrane potential and input resistance values were similar to those of the nonneurogenic lines. Intercellular ionic coupling was consistently observed between cells of a fibroblastoid shape or cells bearing multiple cytoplasmic processes (*i.e.*, all neurogenic lines HV1C, BICR/M1R_K, and 3T3). Epithelioid cells (*i.e.*, HeLa, REe, an NV1C subpopulation, and a GV1C1 variant) showed no such intercellular communication.

In vivo monolayer cultures on glass coverslips were obtained by a modified i.p. diffusion chamber technique. Under these conditions, the cells (with the exception of a glioma-derived cell line) retained the morphological appearance and electrophysiological properties observed *in vitro*.

INTRODUCTION

A single pulse of ENU,[5] administered to rats at the perinatal age, specifically induces malignant tumors in the central and peripheral nervous system (11, 22, 36). Some of the molecular and cellular mechanisms involved in the process of carcinogenesis by ENU have been investigated (3, 10–12). We have recently described an experimental system where, after transplacental exposure to ENU *in vivo* and subsequent transfer to long-term cell culture, FBC (18th day of gestation) underwent neoplastic transformation *in vitro* (25, 26). The morphologically transformed FBC obtained in this "*in vivo-in vitro* system" were tumorigenic upon reimplantation into isogeneic hosts. They have given rise to a variety of heterogeneous or cloned neurogenic cell lines (26).[6] Similar to cell lines previously derived from autochthonous ENU-induced neuroectodermal BD IX rat tumors[6] (26), these lines are aneuploid (14, 24) and express, to varying degrees, the acidic S-100 protein. The S-100 protein is a biochemical marker for cells of the postnatal (15) nervous system, probably mainly for glial cells (Ref. 4; but see Ref. 33). When assayed for neurotransmitter enzyme activity, the neurogenic lines gave mostly negative, in a few instances borderline, results.[6]

In the present study we compare 11 neurogenic with 5 nonneurogenic cell culture lines of different origin, with respect to their electrophysiological properties (*i.e.*, membrane potential, input resistance, electrical membrane excitability, and intercellular ionic coupling). Measurements were carried out both in confluent monolayer cultures *in vitro* and, in parallel, in cells of the same lines cultured on glass coverslips in ID-chambers *in vivo*. Membrane excitability, if demonstrable, would have indicated expression of neuronal properties in the neoplastic cells. Ionic coupling between neurogenic cells was investigated in view of current interest in the absence or presence of intercellular communication in neoplastic cell systems (19, 21, 28, 34).

MATERIALS AND METHODS

Animals

BD IX rats (5) (3 to 5 months old), and in some cases BALB/c mice, were used as hosts for the ID-chamber experiments. The animals were kept on a standard diet (Altromin) and water *ad libitum*.

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[5] The abbreviations used are: ENU, *N*-ethyl-*N*-nitrosourea; FBC, BD IX fetal rat brain cells; ID-chambers, i.p. diffusion chambers.

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Cell Lines

Neoplastic Neurogenic Cell Lines Derived from Neuroectodermal BD IX Rat Tumors

These lines were established in monolayer culture from neuroectodermal tumors in the brain or peripheral nervous system, induced in the offspring of female BD IX rats by an i.v. pulse of 25 μg of ENU per g body weight on the 18th day of gestation. In these animals, death with neuroectodermal tumors occurred at a median age of 240 days ± 20% (S.E.) (26). The histological types of tumors from which the present cell lines (NV1C, NV1Cc, TV1C, GV1C1) were derived are listed in Table 1 along with the proliferative properties and modal G_1-DNA content [as measured by pulse cytophotometry (24)] of the respective cell lines in monolayer culture at the time of the present analyses.

Neoplastic Neurogenic Cell Lines Derived from FBC in Culture

These lines (BT1C to BT7C) originate from cultures of FBC transferred into long-term monolayer culture at 20 to 90 hr after an i.v. transplacental pulse of 75 μg of ENU per g body weight to the pregnant female (18th day of gestation) (25, 26). After an average period of 199 days ± 14% (S.E.), these cultures underwent malignant transformation (26). Table 1 shows the proliferative properties and modal G_1-DNA content of lines BT1C to BT7C. Some experiments were also carried out with FBC exposed to ENU *in vivo* 1 to 2 weeks after transfer into cell culture, *i.e.*, prior to the acquisition of tumorigenicity.

Nonneurogenic Cell Lines

HV1C. Transplantable bile duct carcinoma was originally detected in a BD IX rat that had carried ID-chambers with human myeloid leukemia cells for 1 year. The tumor is maintained by serial passage in both the ascitic and solid (s.c.) form. The HV1C monolayer cell culture line was established after a few transplant passages. HV1C cells have a fibroblastoid appearance.

BICR/M1R$_K$. This neoplastic cell line in monolayer culture was derived from the BICR/M1R transplantable mammary tumor (32), a subline of the BICR/M1 mammary tumor of the Marshall rat (35). The cells are of the fibroblastoid type.

REe. This cell line was derived from Sprague-Dawley rat embryo cells spontaneously transformed to permanent growth. REe cells have been cultured in this institute for more than 8 years. They have an epithelioid appearance and do not produce tumors upon reimplantation into isogeneic hosts (21).

3T3. This fibroblastoid cell line derived from mouse embryo was kindly provided by Professor W. Schäfer of this institute.

HeLa. This epithelioid neoplastic cell line derived from human cervix carcinoma was kindly provided by Professor H. Friedrich-Freksa (deceased) of this institute.

Table 1

Tissues of origin, proliferative properties, mean cell diameter, and modal G_1-DNA content of the neoplastic neurogenic cell culture lines analyzed

Cell line	Tissue of origin	T_D[a] (hr)	Maximal cell density (cell sq cm) × 10^{-4}	Mean cell diameter (μm)[b]	Modal G_1-DNA content[c]
Neoplastic cell lines originating from neuroectodermal BD IX rat tumors					
NV1C	Neurinoma of spinal nerve plexus (NV1), BD IX rat	19	92.0	13	~4
NV1Cc (cloned subline of NV1C)	Neurinoma of spinal nerve plexus (NV1), BD IX rat	29	24.0		~2
TV1C	Neurinoma of Nervus trigeminus (TV1), BD IX rat	29	51.3	15	~2
GV1C1	Mixed glioma (GV1), BD IX rat	21	44.1	8	~2
Neoplastic lines from BD IX FBC in culture					
BT1C	BD IX FBC	32	14.9	8	~2
BT2C	BD IX FBC	30	15.0	8	~4.2
BT3C	BD IX FBC	33	12.4	16	~6.4
BT4C	BD IX FBC	22	32.7	14	~3
BT5C	BD IX FBC	33	24.8	13	~3.4
BT6C	BD IX FBC	26	33.9	14	~3
BT7C	BD IX FBC	37	26.7	15	~2.6

[a] T_D, average population-doubling time in monolayer culture.

[b] Estimated from light-scattering distributions recorded with a cytograph (Bio/Physics Systems Inc.). The values should only be taken as very rough estimates due to limitations of the method.

[c] Modal G_1-DNA content. Relative DNA content per nucleus in the G_1 phase of the cell cycle measured by pulse cytophotometry, in relation to diploid rat leukocytes. This value does not strictly correspond to the chromosome number (cf. Ref. 24 for details). The value 2 corresponds to a diploid DNA content. Values of the modal G_1-DNA content for smaller subpopulations of cells contained in the cell lines are not given.

Cell Culture Conditions

Cells were cultured in Falcon plastic dishes (Falcon Plastics, Oxnard, Calif.) at 37°, using modified (6) Eagle-Dulbecco's medium supplemented with 10% bovine serum, under an atmosphere of 5% CO_2 in air. The medium was renewed at 2- to 3-day intervals, and cultures were passaged at the end of log-phase growth by trypsinization [0.25% trypsin in Ca^{2+}-Mg^{2+}-free isotonic phosphate-buffered saline (137 mM sodium chloride, 2.7 mM potassium chloride, 8 mM dibasic sodium phosphate, 0.5 mM calcium chloride, 0.5 mM magnesium sulfate, and 1.5 mM monobasic potassium phosphate; pH 7.2 to 7.4)]. Electrical measurements were performed on cells in dense monolayers in 50-mm Falcon dishes.

Estimates of average cell diameters of trypsinized cells suspended in phosphate-buffered saline were obtained from recordings of light-scattering distributions, using a cytograph (Bio/Physics Systems Inc., Mahopac, N. Y.). Standard polystyrene particles (diameters, 2, 8 and 14 μm) were used for instrument calibration.

Diffusion Chamber Culture *in Vivo*

ID-chambers were made from acryl plastic rings with a Millipore filter (Millipore GmbH, Neu-Isenburg, Germany; pore size, 0.22 μm) on either side (23, 29). As shown in Fig. 1, a square coverslip glass (previously rinsed in acetone, incubated in 0.25% trypsin overnight, and thereafter rinsed several times in tap and distilled water) was fixed in the middle of the plastic ring with a drop of Tensol glue (I.C.I., Welwyn Garden City, Hertfordshire, England) in each corner. The cells were pipetted into the chambers through a hole in the plastic ring, and the chambers thereafter were sealed with a plastic stopper and Tensol and implanted into the abdominal cavity of BD IX rats (2 to 4 chambers/animal), as previously described (23). ID-chambers with HeLa and 3T3 cells (1/animal) were implanted into BALB/c mice. After 1 to 4 days, the coverslips bearing monolayers of cells were removed from the chambers for the electrical measurements. For chamber to chamber passages, the cell clots were dissolved with 0.5% Pronase (Serva GmbH, Heidelberg, Germany) and 5% Ficoll (Pharmacia Ltd., Uppsala, Sweden) in 0.9% aqueous NaCl solution under sterile conditions and were reimplanted.

Electrical Measurements

Glass microelectrodes were filled with 3 M KCl plus 2 mM potassium citrate and selected for their resistances (30 to 50 MΩ) and tip potentials (≤ -5 mV in Eagle-Dulbecco's medium). They were connected to calomel electrodes through a 3 M KCl solution. To provide a symmetrical circuit, the indifferent electrode was also a calomel electrode connected to the medium by a Ringer-agar bridge. Membrane potentials (inside negative) were measured by Keithley 605 negative capacitance electrometers (Keithley Instruments, Cleveland, Ohio) and were recorded by a Tektronix 506 oscillograph (Tektronix Inc., Beaverton, Oreg.) or a Graphirac pen recorder (Sefram, Paris, France). For determination of intercellular ionic coupling, hyperpolarizing current pulses of different duration were supplied from a type 160/161/162 Tektronix generator via a photon-coupled isolator (Ref. 2; see Ref. 17 for further details). Determination of the ohmic resistance of the cell membrane (input resistance) can be carried out with a single recording electrode by differentiation of a ramp signal. This technique was used as an additional "quick test" for the presence of intercellular ionic coupling. Because of the larger surface membrane area involved in the measurement, cells always possess a lower input resistance in the coupled than in the isolated state (21). The electrical membrane excitability was tested by depolarizing current pulses of 1-msec duration and different amplitudes.

Coverslips with cell monolayers from ID-chambers were transferred to Petri dishes containing either Eagle-Dulbecco's medium or cell-free ascites fluid obtained from BD IX rats after 2 injections of 0.25% polyvinylpyrrolidone (Serva GmbH) in 0.9% aqueous NaCl solution. Electrical measurements on the cells cultured *in vivo* could then be performed in the same way as on the cells cultured *in vitro*. All electrical measurements were carried out at room temperature under a Standard RA microscope (Carl Zeiss, Stuttgart, Germany) with a 40× water immersion objective.

RESULTS

Neoplastic Neurogenic Cell Lines Cultured *in Vitro*. All cell lines derived from ENU-induced neuroectodermal tumors *in vivo* were maintained as monolayer cultures. Their cellular morphology was of the fibroblastoid type. Some of their proliferative properties (*i.e.*, population-doubling time, T_D, and maximum cell density) as well as their fluorometrically measured G_1-DNA content are shown in Table 1. Electrical measurements were performed on cells in dense monolayers. This usually results in more stable membrane potentials and permits reliable measurements of intercellular ionic coupling (17).

The membrane potentials of these lines ranged from 25 to 45 mV (Table 2). There was no evidence for electrical membrane excitability in >400 analyzed cells. In general, all lines exhibited intercellular ionic coupling (Table 2). However, the neurinoma-derived line NV1C (but not its cloned subline NV1Cc) contained a subpopulation (termed Subpopulation a in Table 2) of rounded cells which showed no intercellular ionic coupling, neither among themselves nor with cells of Subpopulation b. These cells, therefore, had a higher input resistance (10.6 MΩ) than the coupled cells of Subpopulation b (4.5 MΩ). The input resistance, however, also depends on the surface area of the impaled cell. This is indicated, *e.g.*, by the high value (6.5 MΩ) for the GV1C1 cells which are very small (~8 μm diameter) and the low value (2.1 MΩ) for the BT3C cells which are much larger (~16 μm). On the other hand, cells with a high communication ratio (BT5C) had a lower input resistance than cells of the same diameter do, but with a reduced communication ratio (BT4C and BT6C). Therefore, this method is reliable for ionic coupling measurements only when single and monolayer cells can be compared (21). A measurement of ionic coupling with 3 electrodes is demonstrated in Chart 1.

Table 2

Membrane potential, (inside negative), input resistance, and intercellular ionic coupling in neoplastic, neurogenic, and nonneurogenic cell lines in culture

Cell line	Inside negative membrane potential (mV)	N^a	R^b (MΩ)	N^a	Ionic coupling
Neoplastic cell lines originating from neuroectodermal BD IX rat tumors					
NV1C	43.3 ± 0.7[c]	88	6.1 ± 0.1	106	±
NV1C Subpopulation a			$R \geq 8$: 10.6 ± 0.2	28	−
NV1C Subpopulation b			$R < 8$: 4.5 ± 0.2	78	+
NV1Cc[d]	44.9 ± 0.6	117	3.1 ± 0.1	133	+
TV1C	25.1 ± 1.2	71	3.3 ± 0.3	70	+
GV1C1	26.4 ± 1.1	101	6.5 ± 0.4	41	+
GV1C1[xx]	24.7 ± 1.0	42	19.6 ± 0.3	17	−
Neoplastic cell lines derived from BD IX FBC cells in culture					
BT1C	33.3 ± 0.8	87	2.8 ± 0.2	87	+
BT2C	24.6 ± 0.6	74	4.4 ± 0.4	73	+
BT3C	59.5 ± 1.0	82	2.1 ± 0.1	84	+
BT4C	38.1 ± 1.3	52	4.1 ± 0.3	52	+
BT5C	60.7 ± 0.6	170	2.1 ± 0.2	154	+
BT6C	23.5 ± 1.0	77	4.6 ± 0.4	75	+
BT7C	19.2 ± 0.9	97	5.3 ± 0.4	91	+
Established cell lines of nonneurogenic origin[f]					
HV1C	41.9 ± 0.8	70	4.1 ± 0.2	66	+
BICR/M1R_K	52.5 ± 0.3	1013	4.5 ± 0.1	39	+
REe	42.5 ± 0.5	374	11.6 ± 0.4	65	−
3T3	31.8 ± 0.7	201	6.3 ± 0.4	71	+
HeLa	29.5 ± 0.7	140	16.3 ± 0.7	67	−

[a] *N* refers to the total number of cells measured at confluency in 4 different culture dishes.

[b] Note the high input resistance *(R)* of the cells that were not ionically coupled (NV1C, $R \geq 8$; GV1C1[xx]; REe; HeLa).

[c] Mean ± S.E.

[d] Cloned subline of NV1C.

[e] Morphological variant of the GV1C1 line, with drastic change from spindle-like to rounded shape.

[f] Data in part compiled from studies published elsewhere (16, 20, 21).

A 30-na current pulse of 25 msec resulted in a hyperpolarization of V_1 = 40 mV in the NV1C cell impaled with both the recording and the stimulating electrodes. In a cell separated from the first one by at least 1 other cell, a hyperpolarization of V_2 = 30 mV was still registered. During the first few passages after transfer to cell culture, the primary glioma-derived GV1 cells appeared rather density inhibited (Fig. 2*a*). At this stage, cells were very sensitive to electrode impalement and only a few of them survived this procedure. In contrast, primary cells from GV1 glioma reached a much higher density in ID-chambers, with long, spindle-like cellular morphology (Fig. 2*b*). During subsequent passages *in vitro*, the cells began to proliferate with a regular parallel arrangement, now with the same long, spindle-like appearance as previously seen in the ID-chamber cultures (Fig. 2*c*). At this stage, membrane potentials, input resistances, and intercellular ionic coupling were recorded (Table 2). After a number of further passages, the GV1C1 line produced a morphological variant (GV1C1[xx]; Fig. 2*d*), which could later also be established as a suspension culture line in spinner flasks (GV1C2). The GV1C1[xx] cells were rounder and, correspondingly, much less adherent to the dishes. They were no longer ionically coupled, but their membrane potentials remained unchanged (Table 2).

The cell lines derived from FBC that had undergone neoplastic transformation in culture after exposure to ENU *in vivo* showed electrophysiological properties similar to the properties of tumor-derived neurogenic lines. All of the FBC-derived lines showed a fibroblastoid cellular morphology and membrane potentials ranging from 19 to 60 mV (Table 2). No membrane excitability could be detected in 600 analyzed cells. All lines showed intercellular ionic coupling. There was an indication that cell lines with higher membrane potentials were ionically coupled with a higher communication ratio V_2/V_1 [defined as the ratio of the voltage change V_2 in the coupled cell to the voltage change V_1 in the cell containing both the recording and the stimulating electrodes (17)]. This was also indicated by their somewhat lower input resistance (Table 2). No tendency for a correlation between membrane potentials, doubling time, and maximal cell density could be detected.

In Chart 2, the recorder diagram of a coupling experiment with BT5C cells is demonstrated. Beginning on the right

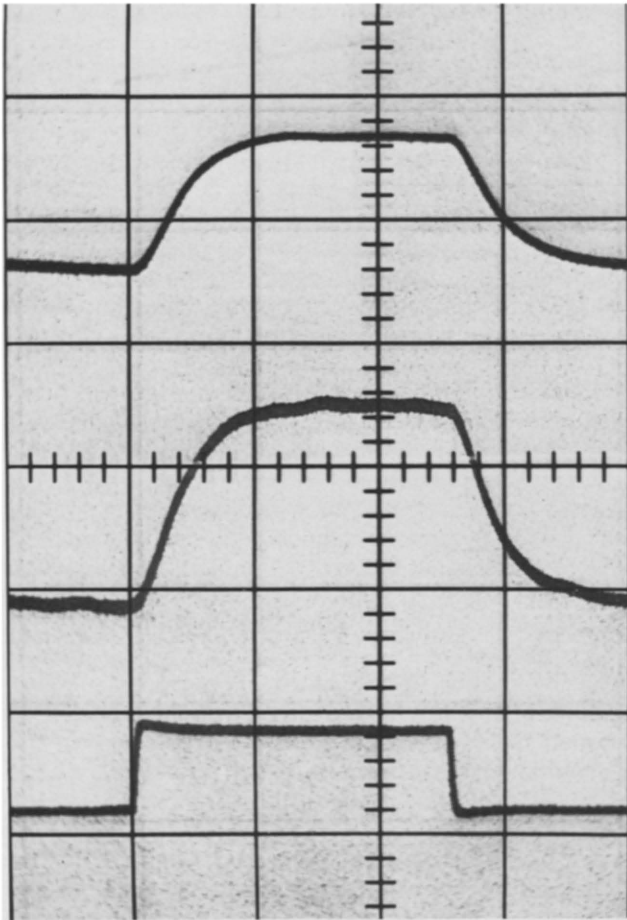

Chart 1. Ionic coupling between NV1C cells (Subpopulation b). A 30-na current pulse of about 25-msec duration (*lower trace*) results in a hyperpolarization of about 40 mv in the cell with the inserted stimulating electrode (*middle trace*) and in a hyperpolarization of about 30 mv in another cell of the monolayer (*upper trace*).

side, the 2 potential base lines can be seen. After 2 very distant cells were impaled with the recording electrodes, membrane potentials of −70 mV were registered. Attempts to insert the stimulating electrode into the cell represented by the lower trace are reflected by instabilities of the membrane potentials occurring synchronously in both cells (recorder traces are separated by 24 sec on the time axes). Successful insertion of the stimulating electrode is indicated by 10-sec pulses of about 10 and 5 mV, respectively, in the 2 impaled cells. Withdrawal of the stimulating electrode resulted in a transient drop of the membrane potential. Insertion of this electrode into the other cell gave the same communication ratio, indicating that no rectification took place in these cells. After the stimulating electrode was withdrawn, almost the original membrane potentials reappeared, and withdrawal of both recording electrodes resulted in the registration of the original base lines.

Intercellular ionic coupling was also found in untreated FBC cultures and in FBC cultures previously exposed to ENU *in vivo* when measured after 1 to 2 weeks of culture [*i.e.*, prior to the acquisition of tumorigenicity; (26)]. Both the untreated and ENU-pretreated FBC were very sensitive to electrode impalement and became leaky in many cases. This and the limitations of cells at this stage prevented precise determination of their membrane potentials and input resistances. At successful impalements, however, values in the same range as in the permanent lines were recorded.

The membrane potentials of the nonneurogenic cell lines were in the same range as those of the neurogenic lines (Table 2). Intercellular ionic coupling was always found in the 3 fibroblastoid lines (HV1C, BICR/M1R_K, 3T3). Both epithelioid lines (REe, HeLa) were not ionically coupled. Again, the lack of ionic coupling in these lines was reflected by an increased input resistance (Table 2).

Cell Lines Cultured on Coverslips in ID-Chambers *in Vivo*. Cells of the GV1 glioma (1st s.c. transplant passage) were cultured on coverslips in ID-chambers directly from the primary tumor cell suspension prepared to establish the GV1C lines *in vitro*. Similarly, the neurinoma-derived line NV1C and line BT5C were cultured in ID-chambers, and their properties were compared with cells cultured *in vitro* in parallel. Of the nonneurogenic cell lines, BICR/M1R_K, REe, 3T3, and HeLa cells were investigated under both culture conditions.

All cell lines formed monolayers on the coverslips when the ID-chambers were kept in the peritoneal cavity for several cell generations (1 to 3 days). The coverslips were generally surrounded by cell and fibrin clots containing most of the cells. The proliferative properties of some of these cell lines in ID-chambers have been described elsewhere (23).

With the exception of the primary GV1 cells mentioned above, no difference in cellular morphology could be found when a given cell line was either cultured *in vitro* or *in vivo*. As an example, the fibroblastoid BICR/M1R_K cells are

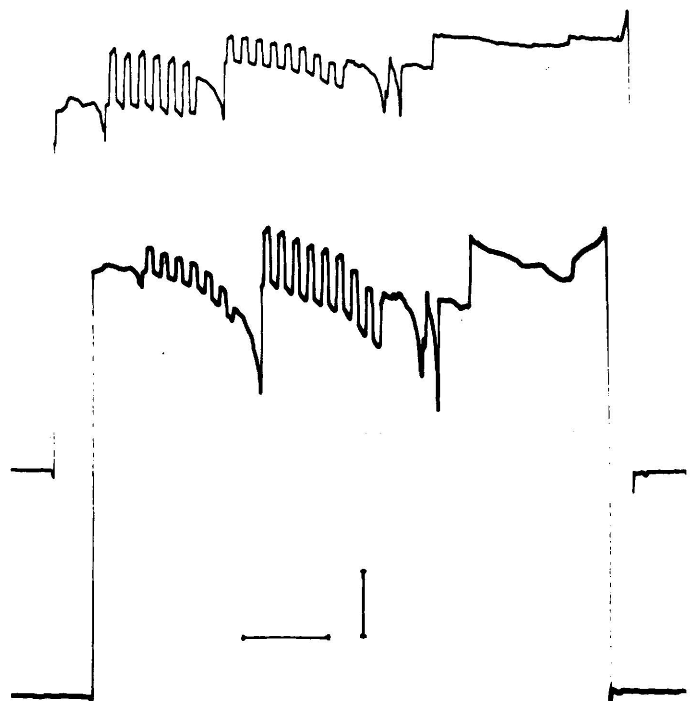

Chart 2. Recorder diagram of a coupling experiment between BT5C cells. *Horizontal bar*, 1 min; *vertical bar*, 10 mv. For further explanation see text.

shown under both types of culture conditions in Fig. 3. The epithelioid HeLa and REe cells (not ionically coupled *in vitro*) remained uncoupled in the ID-chambers. The fibroblastoid lines HV1C, BICR/M1R_K, and 3T3 (ionically coupled *in vitro*) showed intercellular ionic coupling also when grown in ID-chambers. Ionic coupling was equally observed when the culture medium was replaced by BD IX rat ascitic fluid for the measurements, in order to imitate microenvironmental conditions in the peritoneal cavity. With the exception of the GV1C cells, the membrane potentials of cells cultured in ID-chambers were slightly lower than those of the same cells *in vitro*.

DISCUSSION

In this study we have attempted to characterize, with respect to their electrophysiological properties, a number of recently established neurogenic rat cell lines (26).[6] These were either derived from ENU-induced neuroectodermal BD IX rat tumors (NV1C, NV1Cc, GV1C1, GV1C1^{xx}) or obtained in an *in vivo-in vitro* system (25, 26) where FBC exposed to ENU *in vivo* and subsequently transferred into long-term cell culture underwent malignant transformation *in vitro* (BT1C to BT7C). For comparison, several nonneurogenic cell culture lines of either fibroblastoid (HV1C, BICR/M1R_K, 3T3) or epithelioid cellular morphology (REe, HeLa) were included in the analysis.

In the search for information on the type(s) and differentiated state(s) of those cells, among the different subpopulations of FBC, that underwent malignant transformation both *in vivo* and in the *in vivo-in vitro* system, the absence of membrane excitability in the neurogenic lines was of particular interest. Membrane excitability of cultured cells may escape detection by depolarizing current pulses, if accommodation has been obtained. This could be the case with cells adapted to a low membrane potential, *e.g.*, due to environmental conditions in culture. Such cells would, therefore, no longer be excited by depolarization; however, excitability would be restituted by hyperpolarization. The cell lines analyzed in this study did not show this effect, as indicated by the experiments of ionic coupling. The hyperpolarizing current pulses of 25 msec as well as of 10-sec duration used in the latter experiments, in no instance evoked action potentials.

The absence of membrane excitability, the lack of (or borderline) neurotransmitter enzyme activities[6] (26), and the presence (to a varying degree and in varying proportions of cells) of the S-100 protein (26)[6] suggest that phenotypically most (if not all) of the cells in these lines bear traits of glial, but not of neuronal lineages. This may seem surprising, since (*a*) neurons and neuroglia are believed to possess common precursors (27) and (*b*) the FBC initially exposed to the ENU pulse should have contained cells of both the glial and neuronal lineages (9, 26). In principle, therefore, occurrence of neoplastic cells expressing glial and/or neuronal properties could have been expected. On the other hand, it is possible that the probability of malignant transformation may depend on the differentiative stage of the target cells at the time of interaction with a carcinogen (11, 26, 30, 31) and that the "risk" of transformation might decrease with the progression of cells through the precursor stages toward a terminally differentiated, nonproliferative end state.

During rat brain development, and on an overall scale, the neuronal system precedes the glial cell populations with regard to proliferation and differentiation (1). At the stage of brain development chosen for the ENU pulse (18th day of gestation), the precursor compartments would be expected to contain predominantly cells committed for glial lineages. This, in turn, could have reduced the transformation frequency of neuronal precursors to a low level. Interestingly, some neoplastic cell lines expressing neuron-like properties have recently been derived from neuroectodermal BD IX rat tumors induced by transplacental exposure to ENU on the 15th day of gestation (Ref. 33; see also Ref. 13).

Membrane potentials and input resistances were in the same range in the tumor-derived neurogenic lines and those obtained by the *in vivo-in vitro* procedure, in the cultures of both untreated FBC and treated FBC at 1 to 2 weeks after exposure to ENU *in vivo*, and in the nonneurogenic lines analyzed for comparison. Both cellular morphology and electrophysiological parameters did not significantly differ when the same cells were cultured either on a plastic surface *in vitro* or on glass coverslips in ID-chambers.

Like the normal FBC cultures, most neoplastic neurogenic lines exhibited intercellular communication, as demonstrated by ionic coupling via low-resistance junctions between cells. These low-resistance junctions are considered to be identical with the "gap-junctions" demonstrated by electron microscopy (8, 18). Lack of intercellular ionic coupling (as an expression of impaired intercellular communication and control) had earlier been invoked as a general feature of neoplastic cells (28). However, in the meantime, numerous exceptions to this notion have been reported for both neoplastic tissues (19, 34) and malignant cell populations in culture (7, 21). Rather, the presence or absence of intercellular ionic coupling seems to be dependent on particular structural membrane differences associated in a still unclarified manner with a fibroblastoid *versus* epithelioid morphological appearance of permanently cultured cells (16, 17, 21). Established fibroblastoid cells (*i.e.* spindle-shaped cells and cells bearing cytoplasmic processes like the present neurogenic cell lines BT1C to BT7C) appear to be mostly ionically coupled, while established epithelioid cells (*e.g.*, the lines REe and HeLa in this study) are not. This observation has been confirmed by the data reported here for cultured neoplastic cells grown both *in vitro* and on glass coverslips from ID-chambers *in vivo*. An interesting example in this context was the change from an ionically coupled to a noncoupled state, coinciding with the appearance of the round morphological variant GV1C1^{xx} in the fibroblastoid line GV1C1.

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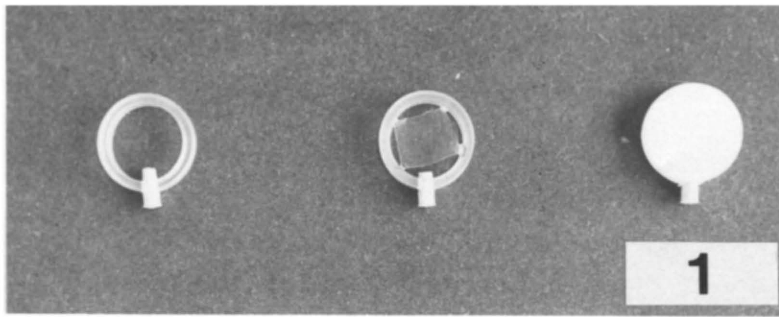

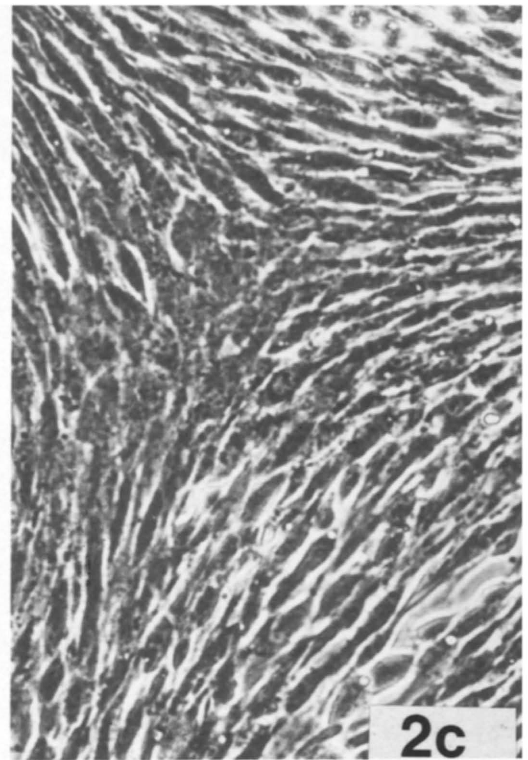

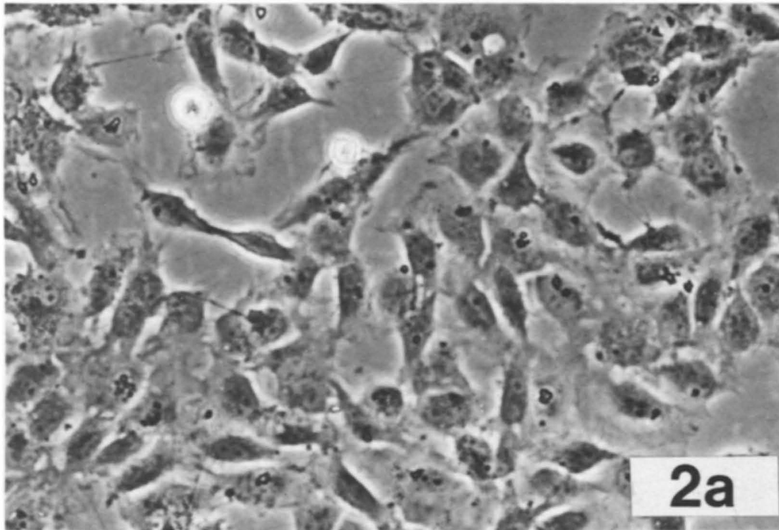

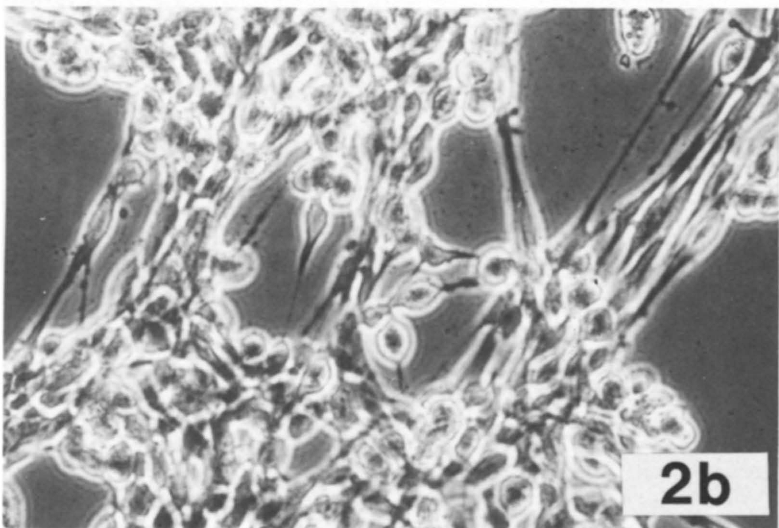

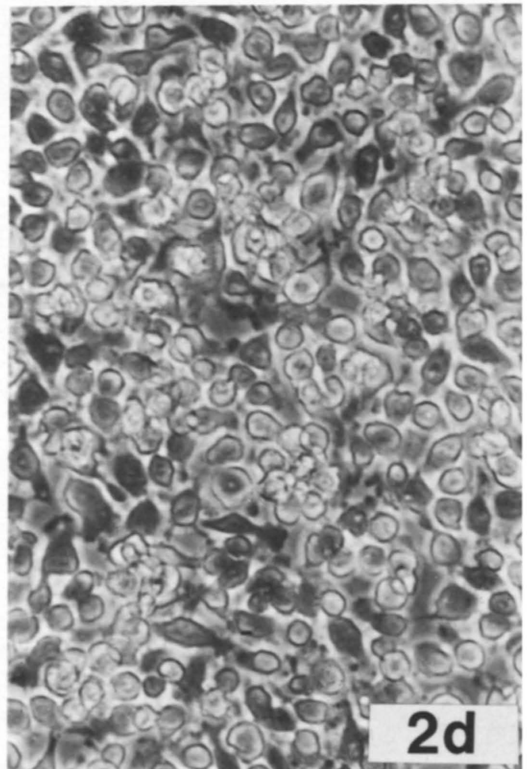

Fig. 1. Preparation of diffusion chambers for monolayer culture *in vivo*. *Left*, an acryl plastic ring (outer diameter, 13 mm) with plastic stopper; *middle*, coverslip glass fixed in the plastic ring; *right*, complete chamber with a Millipore filter on each side.

Fig. 2. GVIC glioma cells. *a*, primary culture *in vitro*; *b*, the same primary culture in diffusion chamber *in vivo*; *c*, cells after more than 2 months *in vitro* (6th passage); *d*, cells after more than 6 months *in vitro* (40th passage). Phase contrast, × 400.

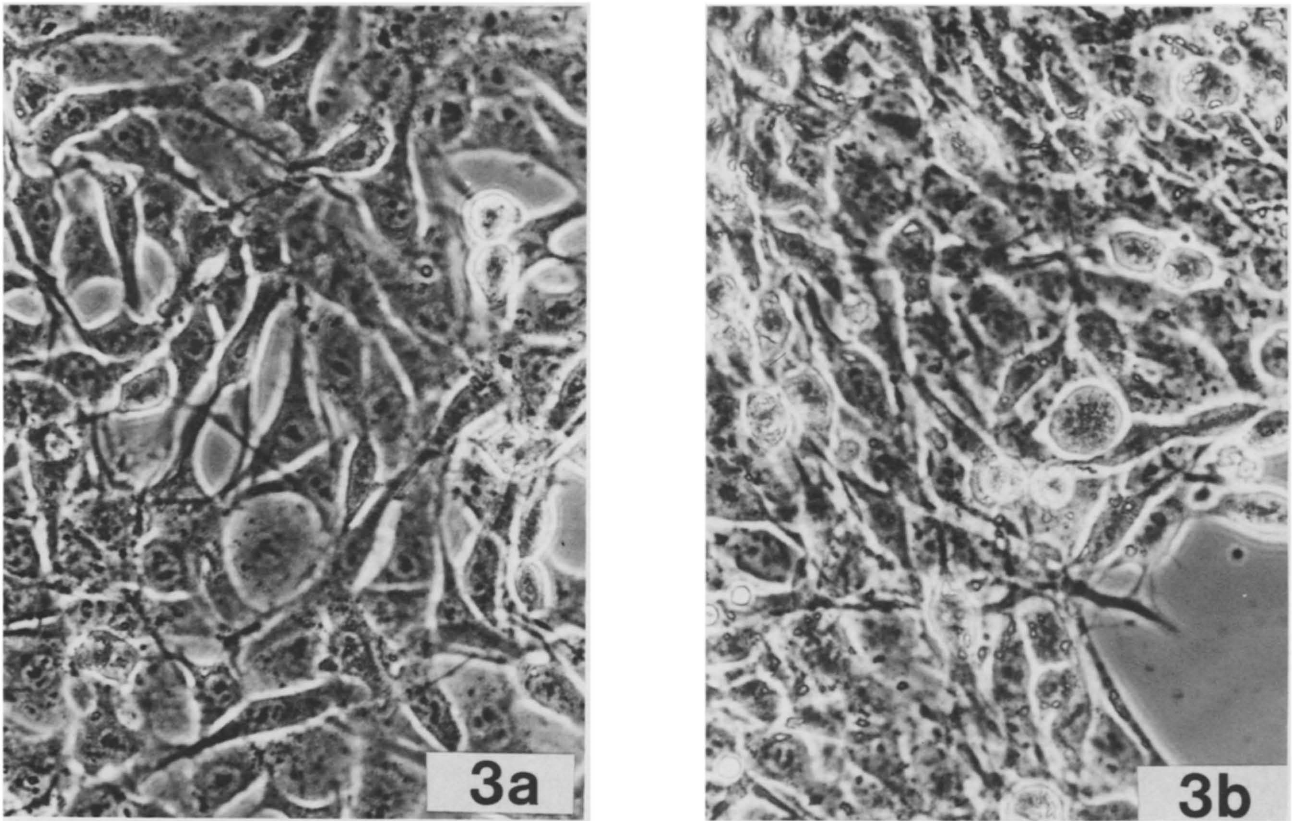

Fig. 3. BICR/MIR$_K$ mammary tumor cells. *a*, cells grown *in vitro*; *b*, cells grown in ID-chamber *in vivo* showing identical morphology. Phase contrast, × 400.