the axonal development of identified cells in many different species shows that specific neurons recognize specific central axon tracts and regions of the neuropil. These studies suggest that a process of selective fasciculation mediated by cell contact is responsible for the specific arrangement of neuronal processes. In recent work, it has been shown that the filopodia of individual neurons can correctly distinguish certain axon fascicles (*20*).

Our data provide direct evidence for the presence of chemical differences on the surfaces of axon fascicles in the adult and in the embryo. These antigenic differences are present on the filopodia and are carried on a family of protease-sensitive, high molecular weight glycoconjugates. We do not yet know whether the particular antigens we have described are themselves directly involved in the mechanisms that guide selective axon fasciculation, but the fact that these molecular differences are present from early embryonic stages is consistent with the possibility that molecular differences between axon fascicles are responsible for the elaborate and precise geometry of axon outgrowth.

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Modulation of Synapse Formation by Cyclic Adenosine Monophosphate

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How neurons in the developing nervous system form synapses and distinguish appropriate from inappropriate synapses remains one of the central, unsolved problems in neurobiology. In 1963, Sperry (*1*) proposed the chemoaffinity hypothesis; namely, that neurons bear positional labels (that is, molecular addresses) that are recognized by complementary molecules on the synaptic target cells and thereby determine the specificity of neuronal connections. He also suggested that two gradients of molecules on retina neurons at right angles to one another, which interact with complementary molecules on the target neurons in the tectum, might be a mechanism for matching synaptic connections and reproducing a point-to-point map of the retina in the tectum. If synapse recognition molecules exist, monoclonal antibody technology should be a powerful tool for their detection. Many investigators are now using this approach.

Other mechanisms such as regulation of gene expression by environmental factors such as hormones, neuromodulators, transynaptic communication, or molecules secreted by neighboring or other cells surely play important roles in the assembly of synaptic circuits. For example, Le Douarin (*2*) and Patterson (*3*) and their colleagues have shown that during development neurons from the neural crest can express either the gene for tyrosine hydroxylase, which catalyzes the first step in the pathway for norepinephrine synthesis, or the gene for choline acetyltransferase, which catalyzes the synthesis of acetylcholine, depending on the presence of an extracellular macromolecule, purified by Weber (*4*), which is secreted by other cells, or the extent of depolarization of the neuron. In addition, Mudge (*5*) has shown that the expression of somatostatin, a peptide transmitter or neuromodulator, by dorsal root ganglia sensory neurons is dependent on molecules secreted by nonneural cells. Raff *et al.* (*6*) also have shown that fetal calf serum markedly influences the differentiation pathway expressed by glial cells in the central nervous system.

Edelman and his colleagues (*7*) discovered a neuronal glycoprotein rich in sialic acid residues, termed N-CAM (neural cell adhesion molecule), that mediates intercellular adhesion in the absence of Ca^{2+} and probably plays an important role in the development of the nervous system by conserving the topographic relationships between individual neurons or axons (or both) in a set of neurons, even though axons may migrate long distances before synapsing. Molecules that mediate Ca^{2+}-dependent intercellular adhesion (*8*) and factors that promote retina cell adhesion, such as cognin (*9*), and ligand and agglutinin (*10*), also have been described, but little is known about their function in the nervous system. Other mechanisms such as contact guidance, chemotaxis, cell survival factors, guidance of neurites by glia (*11*), and

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Summary. Synapses between neuroblastoma-hybrid cells and myotubes exhibit a high degree of plasticity. Increase of cyclic adenosine monophosphate (AMP) levels of the hybrid cells for several days results in the appearance of functional voltage-sensitive Ca^{2+} channels, which are required for evoked secretion of acetylcholine. The results show that cyclic AMP regulates synaptogenesis by regulating the expression of voltage-sensitive Ca^{2+} channels, and suggest that cyclic AMP affects posttranslational modifications of some glycoproteins and cellular levels of certain proteins.

selection for synchronous or sequential transmission across two or more synapses that innervate a neuron may also play important roles in synaptogenesis.

We have used monoclonal antibodies and cultured cell systems to study synapse formation and plasticity. Some studies with retina cells are discussed first, and then studies on the plasticity of synapses formed by clonal neuroblastoma-hybrid cells with striated muscle cells are reviewed.

A dorsal-ventral gradient of protein in retina. Trisler *et al.* (*12*) obtained a monoclonal antibody that recognizes a cell membrane protein distributed in a large dorsal-ventral topographic gradient in chick retina (Fig. 1). The concentration of antigen detected at the dorsal margin was at least 35-fold higher than that found at the ventral margin of the retina, and the concentration of antigen detected varied continuously and logarithmically with the logarithm of distance along the circumference of the retina from ventral to dorsal poles of the gradient. Thus, the protein defines a bilaterally symmetrical, dorsal-ventral axis of the retina and can be used as a marker of cell position in the retina with respect to the dorsal-ventral axis. The antigen, termed TOP (toponimic), was detected on all cells examined in dorsal and middle retina, but more TOP was detected on cells from dorsal retina than on cells from middle retina.

The TOP antigen was solubilized and purified by antibody-agarose column chromatography and sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis. A single band of protein was obtained with a molecular weight (M_r) of approximately 47,000 (*13*). TOP was detected in optic cups of 48-hour chick embryos (*14*), and evidence for a gradient of TOP was found in 4-day embryo retinas. A gradient therefore is generated as neurons are generated in the retina and the gradient is maintained throughout embryonic development and in the adult. Neurons first appear in the central portion of retina and then are added in concentric, ever widening rings. Thus, central retina is the oldest portion of the retina and peripheral retina is the youngest. How a dorsal-ventral gradient is generated as the retina forms and is perpetuated is not known.

TOP was detected, in order of decreasing concentration, in retina, cerebrum, and thalamus; little or no antigen was found in other parts of the nervous system or in other tissues. Gradients of TOP were found in chicken, turkey, duck, and quail retina, but the antigen was not detected in rat, *Xenopus laevis*, *Rana pipiens*, or goldfish retina.

The antigenicity of TOP is destroyed by trypsin; however, cells dissociated with trypsin from dorsal, middle, or ventral retina, cultured separately or combined in various proportions, continue to synthesize the antigen and accumulate the amount of TOP that would be expected with cells from the corresponding region in the intact retina in ovo. Thus, the number of antigen molecules detected on retina cells after 10 days in culture depends on the prior position of the cells in the intact retina.

These results suggest that the retina is composed of a gradient of cells that express different amounts of TOP, depending on the position of the cells in retina along the dorsal-ventral axis of the retina. The function of TOP is not known. Monoclonal antibodies that recognize an anterior-posterior gradient of molecules in retina were looked for, but were not found (*15*). However, the demonstration that TOP is a cell membrane protein and is expressed on the basis of cell position in the retina, rather than cell type, suggests that TOP may play a role in the specification of positional information in the retina. We are trying to clone complementary DNA (cDNA) corresponding to TOP messenger RNA (mRNA) to use to define the amino acid sequence of TOP and to explore the mechanism of regulating TOP expression.

Other monoclonal antibodies to retina. Grunwald *et al.* (*16*) showed that antibody 13H9 recognizes cell membrane protein detected on most or all cells in retina; however, antigen was not detected on neurons or glia in other parts of the nervous system. It is of interest to determine whether the protein specifies a compartment of cells; that is, functions as a cell adhesion molecule that enables retina cells to adhere preferentially to one another rather than to other cells. Three monoclonal antibodies recognize antigens that are restricted to the outer synaptic layer of retina (113F4, 92A2, and 18B8); another antibody (16G6) recognizes antigen in both the inner and outer synaptic layers of retina. Antibody 18B8 binds to glycoproteins and unidentified species of gangliosides (*17*). Other monoclonal antibodies are specific for a single class of cells in retina such as photoreceptors, horizontal neurons, Müller cells, or ganglion neurons, or for a family of cells such as those in the inner nuclear layer of retina. Another monoclonal antibody, A_2B_5 (*18*), recognizes unidentified gangliosides with sialic acid residues and glycoproteins (*17*) that are markers of neurons and some glia (*6, 18*).

Cultured retina cells. Chick retina contains abundant nicotinic and muscarinic acetylcholine receptors that mostly are distributed in layers within the inner synaptic layer of retina (*19*). Cultured neurons dissociated from chick embryo retina also express choline acetyltransferase and acetylcholine receptors, and the neurons form approximately as many synapses in vitro (1.5×10^9 synapses per milligram of protein) as they do in ovo, as judged by electron microscopy (*20*).

The specificity of synapse formation by retina neurons was examined by coculturing dissociated chick embryo or rat retina neurons with inappropriate synaptic partner cells such as striated muscle cells that possess many nicotinic acetylcholine receptors. Retina neurons form functional synapses with most striated muscle cells in 90 minutes, but these synapses are transient and slowly disappear over a period of 5 to 10 days (*21–23*). Cholinergic neurons that are able to synapse with myotubes first appear in chick retina on day 6 of embryonic development, are most abundant on day 8 and comprise approximately 8 percent of the retina cell population, and lose the ability to form synapses with myotubes by day 16 of embryonic development (*23*). However, synapses between retina neurons increase during the culture period and remain abundant after all synapses between retina neurons and muscle cells terminate.

Two processes contribute to the turnover of retina neuron synapses with myotubes. First, retina neurons are able to form synapses with striated muscle cells only for a short time during development (*23*); and second, synapses between retina neurons and myotubes ter-

minate because retina neurons preferentially adhere to other retina cells rather than to myotubes (*21*).

Preparations of neurons from chick embryo spinal cord, which presumably contain motor neurons that normally innervate striated muscle cells, also form synapses with cultured muscle cells, but the number of synapses remains constant during subsequent culture (*22*). Therefore, spinal cord neurons either form stable, long-lived synapses with muscle cells or attain a steady state wherein the rate of synapse formation is equal to the rate of synapse termination. These results show that inappropriate synapses between retina neurons and myotubes form rapidly and are terminated slowly, that synapses formed by cholinergic neurons from retina and spinal cord turn over at different rates, and that differences in synapse turnover rates of two populations of synapses can result in the selective retention of one population and the loss of the other.

Clonal Neuroblastoma Cell Lines

Adult neurons do not divide; however, the establishment of clonal lines of neuroblastoma cells from a transplantable mouse neuroblastoma tumor (C-1300) of spontaneous origin provided a source of relatively homogeneous populations of dividing cells of neural origin (*24*). Characterization of these (*24*) and other (*25*) clonal lines of C-1300 neuroblastoma showed that the cells have excitable membranes (*26*) and other neural properties, and that the expression of genes for neural properties is inherited and thus can be perpetuated. Clonally inherited differences in phenotype also were found; for example, some neuroblastoma cell lines synthesize acetylcholine (*25*), others catecholamines; but most do not synthesize these compounds.

Cells from neuroblastoma lines that synthesize acetylcholine were cocultured with striated muscle cells, which possess abundant nicotinic acetylcholine receptors, or with cardiac muscle cells that have muscarinic acetylcholine receptors. However, for several years we, and others, failed to detect synapses. We thought that these cell lines might not express all genes for proteins that might be required for synaptic communication, and therefore we fused neuroblastoma cells with other cells and generated many somatic hybrid cell lines (*26*). Hybrid cell lines were found that express new neural properties not detected with parental cells (*27, 28*); with other hybrid cell lines some neural properties were extinguished. Eventually five cell lines were found that synthesize acetylcholine and form many synapses with cultured myotubes (*32, 33*). The early attempts to form synapses with neuroblastoma cells failed for two reasons. (i) The extent of neural maturation and ability of cells to form synapses are regulated and are highly sensitive to environmental conditions, making it necessary to find conditions that yield populations of "differentiated" cells. (ii) Most, but not all, of the cholinergic neuroblastoma cell lines that were tested do, indeed, lack reactions that are required for synapse formation. Empirically, we found that populations of neuroblastoma or hybrid cells can be shifted from a poorly differentiated, synapse incompetent state, to a well-differentiated, synapse competent state, by increasing intracellular levels of cyclic adenosine monophosphate (AMP) for days. Selection for nondividing cells also yields well-differentiated populations of cells.

Table 1. Cell line phenotypes [see (*32, 34, 35*)].

Cell lines (No.)	ACh* formation	K^+-Dependent		Vesicles		ACh receptor aggregation protein	Synapse
		$^{45}Ca^{2+}$ uptake	[^{3}H]ACh release	Small clear	Large dense core		
5	+	+++	+++	+	+	+	+++
3	+	+	+	+	+	+	+
2	+	−	−	+	+	+	−
5	+	++	−	+	+	+	− or +
3	+	++	±	+	−	−	− or +
9	−		−				−

*Acetylcholine (ACh).

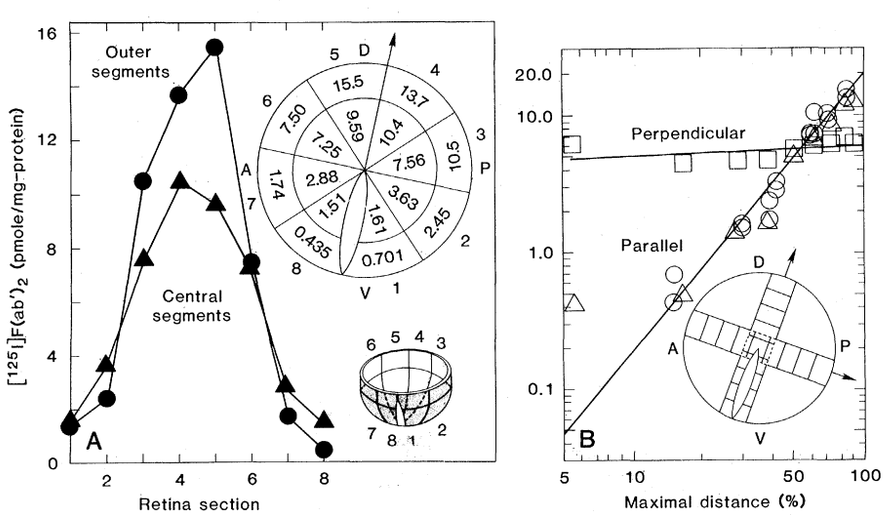

Fig. 1. Geometry of the TOP gradient in 14-day chicken embryo retina (*12*). (A) Specifically bound ^{125}I-labeled F(ab')$_2$ (pmole per milligram of protein) is shown on the ordinates in (A) and (B) and within the appropriate segment of retina tested. (B) The circumference of the retina is 14.5 mm which corresponds to 100 percent on the abscissa. (△) Strips of retina from ventroanterior (0 percent) to dorsoposterior (100 percent) retina margins were removed, and each was cut into nine segments and assayed for TOP. (□) Strips of retina from anterior (0 percent) to posterior (100 percent) margins of the retina perpendicular to the choroid fissure were prepared and assayed as above; (○) data from panel A.

In Fig. 2 are shown photomicrographs of cells from four of the five cell lines that form many synapses with striated muscle cells. The NBr10-A and NBr20-A cells originated by fusion of mouse neuroblastoma N18TG-2 (*26*) with clonal BRL30-E rat liver cells, NCB-20 cells (*29*) resulted from fusion of N18TG-2 cells with fetal Chinese hamster brain cells, and NG108-15 (*30*) resulted from fusion of N18TG-2 with C6BU-1 (*28*) rat glioma cells. Few neurites or synapses were found when cells were in the logarithmic phase of growth. However, exposure of cells for 7 days to 1 m*M* dibutyryl cyclic AMP, which promotes neurite extension, and to 1 percent (rather than 5 percent) fetal bovine serum, which reduces neurite retraction, yields cells with neurites that can be more than 2 mm in length (*31*).

Other cell lines have high concentrations of acetylcholine, adhere well to myotubes, but do not form synapses (*32*). A summary of phenotypes of cell lines with or without synaptic defects is shown in Table 1. The NBr10-A, NBr20-

A, NCB-20, NG108-15, and NS-26 neuroblastoma cells (*25*) form many synapses with cultured myotubes (*32, 33*), synthesize acetylcholine (*32*), have functional voltage-sensitive Ca^{2+} channels (*34*), have small clear vesicles approximately 60 nm in diameter and large dense-core vesicles 180 nm in diameter (*41*), and release acetylcholine into the medium and a protein that stimulates the aggregation of nicotinic acetylcholine receptors on myotube plasma membranes (*35*). Cells from three lines take up Ca^{2+} ions slowly (*34*) and secrete little acetylcholine when depolarized by 80 m*M* K^+ ions, and form few synapses with muscle cells. Cells from two lines lack functional voltage-sensitive Ca^{2+} channels (*34*) and do not form synapses. Cells from five lines take up Ca^{2+} when depolarized by K^+ ions but do not respond by secreting more acetylcholine (*32*), and few or no synapses were found. These cells lack a Ca^{2+}-dependent acetylcholine secretion reaction (or reactions); however, acetylcholine is secreted into the medium in the basal, unstimulated state. Cells from three lines have small clear vesicles but lack large dense-core vesicles and functional protein that induces nicotinic acetylcholine receptor aggregation on myotube membranes (*35*), and form few or no synapses. Nine additional cell lines have little or no choline acetyltransferase activity, and therefore they synthesize little or no acetylcholine (*32*) and do not form functional synapses with striated muscle cells.

Regulation of synaptogenesis. Thus far, we have identified 12 species of receptors that are expressed by NG108-15 cells, including receptors for prostaglandin E_1 (PGE_1) (*36, 37*), prostaglandin F_2 (PGF_2) (*36*), adenosine (*38*), Met-enkephalin (*36*), alpha-2-adrenergic receptors (*39*), depolarizing muscarinic acetylcholine receptors (40), serotonin and LSD receptors (*29*), and receptors for bradykinin, neurotensin, angiotensin II, and somatastatin (*32*), and have defined cell responses to the ligands for these receptors. Some receptors, such as those for PGE_1, mediate activation of adenylate cyclase; other receptors such as Met-enkephalin receptors, muscarinic depolarizing acetylcholine receptors, and alpha-2-adrenergic receptors mediate inhibition of adenylate cyclase.

Increase of cyclic AMP in neuroblastoma or hybrid cells for 5 to 7 days, obtained either by treating cells with PGE_1 to increase the endogenous rate of cyclic AMP synthesis or by inhibition of cyclic nucleotide phosphodiesterase with dibutyryl cyclic AMP, or theophylline, resulted in increases in the percentage of myotubes tested that were innervated and the rate of spontaneous secretion of acetylcholine from NG108-15 cells at synapses (*32*) (Table 2). Presumably, each depolarizing response of a myotube to acetylcholine is due to the spontaneous secretion of acetylcholine from a single NG108-15 vesicle. NG108-15 cells and myotubes were cocultured and treated for 5 to 7 days with the compounds shown; then myotubes were assayed for synapses by intracellular microelectrode recording. Treatment of cells with 1 m*M* dibutyryl cyclic AMP, 1 m*M* theophylline, or 10 μM PGE_1 increased the percentage of muscle cells tested that were innervated from 15 to approximately 60 percent and increased 14- to 20-fold the frequency of spontaneous synaptic responses of myotubes (the miniature end-plate potential frequency). Treatment of cells with 10 μM PGE_1 and 1 m*M* theophylline resulted in innervation of 98 percent of the myotubes tested and increased the frequency of synaptic responses of myotubes 45-fold. No immediate effect of these compounds on the cell membrane potential or rate of acetylcholine secretion was detected. Half-maximal increases in synapses and rate of spontaneous acetylcholine secretion at synapses were observed when cellular cyclic AMP levels were increased for 1 to 2 days; maximal increases were obtained when cells were treated for 3 to 5 days (*32*).

Table 2. Effect of culture conditions on synaptogenesis and acetylcholine secretion by NG108-15 cells. Each value is the mean of values obtained from more than 75 myotubes. [Data from (*32*)]

Culture conditions	Myotubes with synapses (%)	Synaptic response frequency*
Control	15	0.7
1 m*M* dibutyryl cyclic AMP	55	14
1 m*M* theophylline	64	10
10 μM PGE_1	63	11
10 μM PGE_1 + 1 m*M* theophylline	98	32

*The number per minute per myotube.

In other experiments, NG108-15 cells were incubated with PGE_1, theophylline, dibutyryl cyclic AMP, or PGE_1 and theophylline for 5 to 7 days; then the compounds were withdrawn and cells were incubated for an additional 4 to 14 days to determine whether the effects on synapses and acetylcholine secretion were reversible. On withdrawal of the compounds, synapses and acetylcholine secretion gradually returned to control values in 7 to 11 days (*32*). Thus, the effects of the compounds on synapses are expressed slowly and are long-lived.

Cyclic AMP levels of NG108-15 cells increase markedly in the presence of 10 μM PGE_1 and 1 m*M* theophylline and

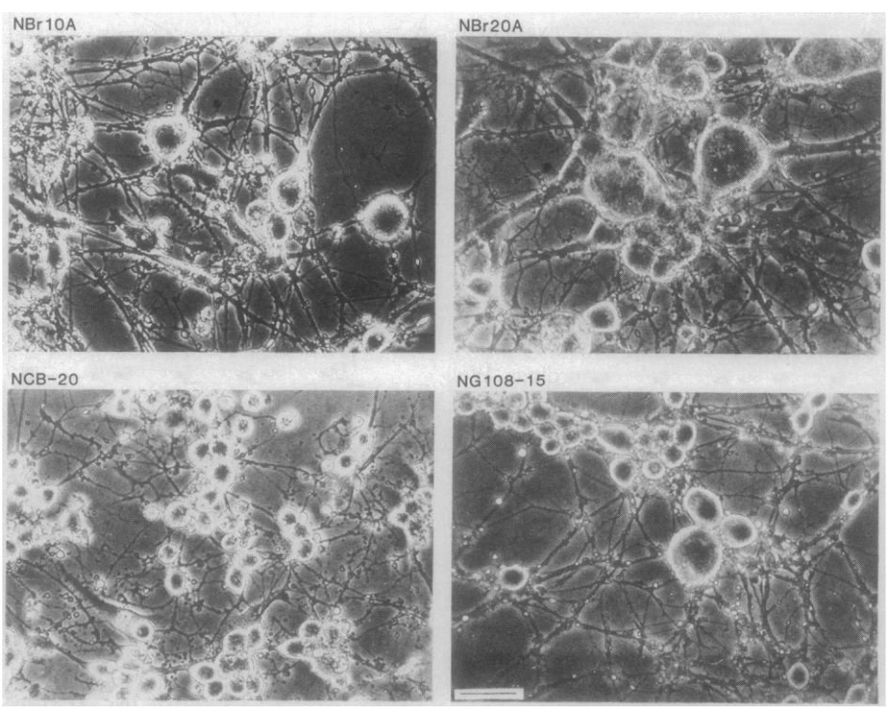

Fig. 2. Neuroblastoma hybrid cells from lines that form many synapses with cultured myotubes were treated for 7 days with 1 m*M* dibutyryl cyclic AMP and the concentration of fetal bovine serum was reduced from 5 to 1 percent between day 5 and day 7. The bar in the lower right hand panel corresponds to 50 μm in each panel. [Data from (*32*)]

remain higher than those of control cells for seven or more days. Intracellular acetylcholine in NG108-15 cells also increases eight- and threefold when cells are treated for 3 days with PGE_1 and theophylline or dibutyryl cyclic AMP, respectively (*32*). NG108-15 cells treated with dibutyryl cyclic AMP (*41*) or PGE_1 and theophylline (*32*) for five or more days contain many large dense-core vesicles and small clear vesicles, whereas control cells contain few vesicles. The cyclic AMP-dependent increase in intracellular acetylcholine is due, at least in part, to an increase in the number of acetylcholine storage vesicles in cells.

Depolarization of NG108-15 or NBr10-A cells with 80 m*M* K^+ ions, in place of 80 m*M* Na^+ ions, has no effect on the rate of acetylcholine secretion by untreated NG108-15 or NBr10-A cells. However, cells gradually are shifted from an unresponsive to a responsive state with respect to depolarization-dependent secretion of acetylcholine when treated for 5 to 7 days with 1 m*M* dibutyryl cyclic AMP or 10 μM PGE_1 and 1 m*M* theophylline. Half-maximal and maximal increases in acetylcholine secretion due to cell depolarization were obtained when NG108-15 cells were treated with 1 m*M* dibutyryl cyclic AMP for 2 and 5 days, respectively (*42*).

Depolarization of nerve terminals is known to activate voltage-sensitive Ca^{2+} channels; Ca^{2+} ions then flow into the cytoplasm of axon terminals and increase the rate of secretion of transmitter at the synapse. We therefore examined the effect of prolonged elevation of cyclic AMP levels of NBr10-A or NG108-15 cells on voltage-sensitive Ca^{2+} channel activity. Four kinds of assays were used (*34*). $^{45}Ca^{2+}$ flux, net uptake of Ca^{2+} by cells was measured with a Ca^{2+} specific electrode, Ca^{2+} fluxes were determined in the presence of murexide by a spectrophotometric assay with a stopped-flow apparatus, and Ca^{2+} action potentials of cells were assayed by intracellular microelectrode recording. We found by each method of assay that logarithmically dividing control cells have little or no voltage-sensitive Ca^{2+} channel activity; however, prolonged elevation of cellular cyclic AMP activation of adenylate cyclase of cells with PGE_1, or by inhibition of cyclic nucleotide phosphodiesterase with dibutyryl cyclic AMP or theophylline, gradually results in the acquisition of functional voltage-sensitive Ca^{2+} channels by cells. Assay of Ca^{2+} action potentials elicited by electrical stimulation of single cells with intracellular microelectric recording showed that most untreated NG108-15 or NBr10-A cells lack functional voltage-sensitive Ca^{2+} channels. However, Ca^{2+} action potentials were found in 100 percent of the cells tested that had been treated for four more days with dibutyryl cyclic AMP.

As shown in Fig. 3, $^{45}Ca^{2+}$ uptake by logarithmically dividing, control NBr10-A cells is not affected by depolarization of cells with 80 m*M* K^+. However, cells that had been treated for 7 days with 10 μM PGE_1 and 1 m*M* theophylline or with 1 m*M* dibutyryl cyclic AMP respond to depolarization by 80 m*M* K^+ with a rapid influx of $^{45}Ca^{2+}$ via voltage-sensitive Ca^{2+} channels (*34*). Depolarization-dependent $^{45}Ca^{2+}$ uptake is inhibited completely by $1 \times 10^{-4} M$ D-600 (half-maximal inhibition was obtained with $9 \times 10^{-7} M$ D-600), an alkaloid known to inhibit voltage-sensitive Ca^{2+} channels and slow Na^+ channels. $^{45}Ca^{2+}$ uptake also is inhibited by La^{3+}, Co^{2+}, and Ni^{2+} ions.

Exposure of NG108-15 cells to PGE_1 increases cellular cyclic AMP levels within seconds; however, no immediate effects of PGE_1, PGE_1 and theophylline, or dibutyryl cyclic AMP on voltage-sensitive Ca^{2+} channel activity were detected. Half-maximal and maximal voltage-sensitive Ca^{2+} channel activity were expressed by cells that had been treated with PGE_1 and theophylline or dibutyryl cyclic AMP for 2 and 4 days, respectively.

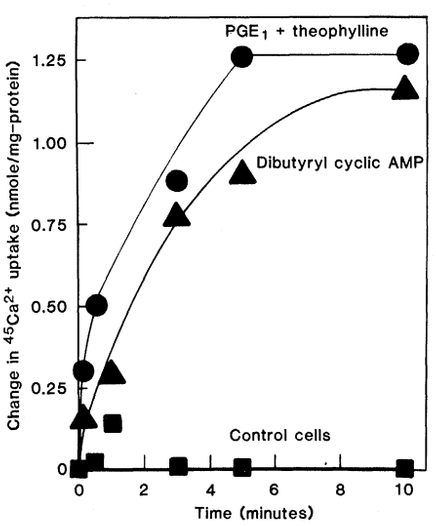

Fig. 3. The effect of culture conditions on the expression of functional voltage-sensitive Ca^{2+} channels of NBr10-A cells. Uptake of $^{45}Ca^{2+}$ due to activation of voltage-sensitive Ca^{2+} channels of untreated logarithmically dividing control NBr10-A cells, cells cultured for 6 days with 1 m*M* dibutyryl cyclic AMP, or 10 μM PGE_1 and 1 m*M* theophylline. The cells were depolarized with 80 m*M* K^+ (in place of 80 m*M* Na^+). Values for $^{45}Ca^{2+}$ binding to cells or uptake at 5.4 m*M* K^+, which were not inhibited by 100 μM D-600 and were not mediated by voltage-sensitive Ca^{2+} channels, were subtracted from the values shown. Uptake of Ca^{2+} dependent on cell depolarization was completely inhibited by 100 μM D-600. [Data from (*34*)]

Relatively weak voltage-sensitive Ca^{2+} channel activity appears in untreated NBr10-A cells when cells form confluent monolayers. Thus, cell concentration or adhesive interactions between cells also regulates the expression of voltage-sensitive Ca^{2+} to some extent.

Nitrendipine and other dihydropyridine derivatives inhibit voltage-sensitive Ca^{2+} channels of smooth muscle (*43*), striated muscle (*44*), and cardiac muscle (*45*), and specific binding sites for ^3H-labeled nitrendipine have been found in these tissues and in brain (*46*). The nitrendipine receptors are thought to be part of the voltage-sensitive Ca^{2+} channel complex, perhaps functioning as regulators of channel activity.

Kongsamut and Müller have shown that $^{45}Ca^{2+}$ uptake by NG108-15 cells mediated by voltage-sensitive Ca^{2+} channels is inhibited by nitrendipine (*47*). We have confirmed this and find that NBr10-A cells are inhibited half-maximally by 3 n*M* nitrendipine. A single class of specific binding sites for ^3H-labeled nitrendipine was found in membranes from NBr10-A cells that had been treated with PGE_1 and theophylline with a dissociation constant, estimated by Scatchard analysis, of $2 \times 10^{-10} M$, which is similar to values reported for other tissues (*43, 45–46*). The maximum number of specific nitrendipine binding sites was estimated to be 61 fmole per milligram of NBr10-A membrane protein, which is equivalent to approximately 16,000 specific sites for nitrendipine per cell. In contrast, few or no specific binding sites for ^3H-labeled nitrendipine were detected in membranes from untreated, logarithmically dividing NBr10-A cells. These results show that cyclic AMP regulates the number of specific nitrendipine receptors per cell. Specific binding sites for ^3H-labeled nitrendipine also were not detected in membranes prepared from two lines of hybrid cells (SB21B-1 and SB37-B) that lack functional voltage-sensitive Ca^{2+} channels and do not synapse with muscle cells.

Cyclic AMP increases the probability of opening Ca^{2+} channels of cardiac muscle cells (*48*); however, responses to cyclic AMP are rapid and thus differ from the slow effects found with NBr10-A cells.

The molecular weights of nitrendipine receptors in intact membranes of smooth muscle (*49*), transverse tubule membranes of skeletal muscle, and cerebral cortex synaptic membranes (*44*) were estimated by radiation inactivation target analysis to be 278,000, 210,000, and

210,000, respectively. Available information suggests that the nitrendipine receptor complex is a glycoprotein with *N*-acetylglucosamine or sialic acid residues (or both) (*50*). Nitrendipine receptors of smooth and cardiac muscle were reported to be covalently labeled with a radioactive affinity label analog of nitrendipine, ^{3}H-labeled 2,6-dimethyl-3,5-dicarbomethoxy - 4 - (2 - isothiocyanatophenyl)-1,4-dihydropyridine; labeled protein then was solubilized and fractionated. A peak of labeled protein with a molecular weight of 45,000 was identified (*49*). These results suggest that the molecular weight of voltage-sensitive Ca^{2+} channel in membranes is 210,000 to 278,000, that each channel is composed of two or more subunits, and that one subunit is a protein with a molecular weight of 45,000, which binds nitrendipine.

NG108-15 cells that had been grown with or without 10 μM PGE_1 were incubated with [^{35}S]methionine to label the protein; the ^{35}S-labeled glycoproteins then were solubilized and fractionated by wheat germ agglutinin-, ricin-, or lentil-lectin column chromatography and by two-dimensional gel electrophoresis (*51*). Elevation of cellular cyclic AMP levels resulted in the disappearance of some ^{35}S-labeled glycoproteins, the appearance of new ^{35}S-labeled glycoproteins with different molecular weights, changes in the apparent abundance of some ^{35}S-labeled glycoproteins, as well as changes in the isoelectric points of other ^{35}S-labeled glycoproteins. A ^{35}S-labeled glycoprotein with a molecular weight of approximately 45,000 was eluted from wheat germ agglutinin–Sepharose with *N*-acetylglucosamine was obtained from cells with high cyclic AMP levels, but was not detected in untreated cells. Twelve ^{35}S-glycoproteins were detected that were expressed by NG108-15 cells with high cyclic AMP levels but not by control cells, and many other ^{35}S-labeled glycoproteins were obtained from PGE_1-treated cells with radioactivities 2.5- to 10-fold higher than those of control cells. These results extend previous reports of differentiation-specific changes in neuroblastoma proteins (*52*).

Exposure of neuroblastoma or hybrid cells to dibutyryl cyclic AMP alters the levels of some species of polysomal mRNA (*53*). Polysomal polyadenylated (poly A^+) RNA from "undifferentiated" and "differentiated" neuroblastoma cells were compared; many species of polysomal poly A^+ RNA were found in RNA from undifferentiated cells, but not differentiated cells (*54, 55*), and conversely, many species of poly A^+ RNA were expressed by differentiated neuroblastoma cells that were not expressed by undifferentiated cells (*55*).

In prokaryotic cells (cyclic AMP · catabolite activator protein) complexes bind to certain sites on DNA and thereby regulate the initiation of transcription of certain genes. Cyclic AMP also regulates the levels of some species of mRNA and protein in eukaryotic cells (*56*), but relatively little is known about the mechanisms of regulation. Cyclic AMP markedly increases the expression of many neural properties in neuroblastoma or hybrid cells, such as voltage-sensitive channels for Ca^{2+}, Na^+, and K^+, and also Ca^{2+}-dependent K^+ channels, neurite extension, vesicles, synapses, acetylcholinesterase, and with some cell lines, choline acetyltransferase, or tyrosine hydroxylase activities. We find that cyclic AMP regulates synaptogenesis, at least in part, by regulating the expression of voltage-sensitive Ca^{2+} channels, which are required for stimulus-dependent secretion of transmitter at synapses. The results suggest that cyclic AMP affects posttranslational modifications of some species of glycoprotein. Appropriate cloned cDNA probes are needed to determine whether cyclic AMP affects the levels of some species of mRNA and to define further the cyclic AMP–dependent mechanisms that affect synapse formation and plasticity.

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