Regulation by Neurotransmitter Receptors of Serotonergic or Catecholaminergic Neuronal Cell Differentiation*

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The murine F9-derived 1C11 clone exhibits a stable epithelial morphology, expresses nestin, an early neuroectodermal marker, and expresses genes involved in neuroectodermal cell fate. Upon appropriate induction, 100% of 1C11 precursor cells develop neurite extensions and acquire neuronal markers (N-CAM, synaptophysin, γ- enolase, and neurofilament) as well as the general functions of either serotonergic (1C11*/5HT) (5HT, 5-hydroxytryptamine) or noradrenergic (1C11*/NE) (NE, norepinephrine) neurons. The two programs are shown to be mutually exclusive. 1C11 thus behaves as a neuroepithelial cell line with a dual bioaminergic fate. 1C11*/5HT cells implement a functional 5-HT transporter and thereby a complete serotonergic phenotype within 4 days, whereas 5-HT1B/D, 5-HT2D, and 5-HT2A receptors are sequentially induced. The time-dependent schedule of catecholaminergic differentiation was defined. Catecholamine synthesis, storage, and catabolism are acquired within 4 days; the noradrenergic phenotype is complete at day 12 and includes a functional norepinephrine transporter and an α1D-adrenoreceptor (day 8). The time-dependent onset of neurotransmitter-associated functions proper to either program is similar to in vivo observations. Along each pathway, the selective induction of serotonergic or adrenergic receptors is shown to be an essential part of the differentiation program, since they promote an autoregulation of the corresponding phenotype.

Immortal cell lines expressing precursor properties and still capable of undergoing neuronal lineage transition may help identify the molecular and cellular events involved in neural differentiation and may help assess the relative contributions of the extrinsic and intrinsic factors involved in the fate of neuronal precursor cells (1). Several strategies have been devised to isolate neural cell lines, including cloning of tumors, targeted oncogenesis in transgenic mice, somatic cell fusion, growth factor-mediated expansion of progenitors of the central nervous system, as well as retroviral immortalization (2). Among these cell lines, of outstanding interest are those that contain the early genes of the simian virus SV40 under the control of the adenovirus E1A promoter, into multipotent cells (7). Due to the low level constitutive expression of the SV40 T antigen, this construct promotes immortalization of neuroectodermal, endodermal, or mesodermal precursor cell lines while still allowing differentiation along multiple pathways to occur (8, 9).

The 1C11 murine cell line, which was selected upon differentiation of F9 multipotent embryonal carcinoma cells transfected with PK4 (7), behaves as a neuronal progenitor. It maintains an undifferentiated phenotype and responds to induction by producing a progeny with neuron-associated markers. 1C11 cells convert within 4 days into serotonergic cells able to metabolize, store, and take up serotonin (5-HT)3 (8, 10) and expressing 5-HT1B/D, 5-HT2A, and 5-HT2B receptors (11).

Additional experiments shown here establish that the selective induction of these three 5-HT receptors is an essential part of the serotonergic program. Indeed, the cells synthesis, storage, and transport of 5-HT respond to 5-HT concentration in the growth medium. The present study also introduces alternative culture conditions capable of triggering the 1C11 clone toward catecholaminergic differentiation. Similar to the serotonergic program, the catecholaminergic pathway recruits nearly 100% of the cells and follows a well defined schedule. Although the enzymes of catecholaminergic metabolism are

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1 The abbreviations used are: 5-HT, 5-hydroxytryptamine; CCA, cyclohexane carboxylic acid; DA, dopamine; BeCAMP, dibutyryl cyclic AMP; HEAT, 2-[4-(4-hydroxy-3-isodophenyl)ethyaminomethyl] tetralone; NE, norepinephrine; NET, NE transporter; TH, tyrosine hydroxylase; IP3, inositol 1,4,5-trisphosphate; PLC, phospholipase C; FCS, fetal calf serum.
fully acquired within 4 days, 12 days of induction are necessary for the cells to display a complete catecholaminergic phenotype, coincident with the induction of a functional noradrenergic (NE) uptake. Screening differentiating catecholaminergic cells to detect bioaminergic receptors revealed the onset of a single adrenergic receptor subtype, α1D, at day 8 of differentiation. This receptor plays a pivotal role in the implementation of the catecholaminergic program, since its inhibition prevents the cells from acquiring a functional NE transport.

**EXPERIMENTAL PROCEDURES**

**Materials**—Dibutyryl cyclic AMP (Bt2cAMP), cyclohexane carboxylic acid (CCA), and dimethyl sulfoxide (Me2SO) were from Sigma-Aldrich. Betaxolol and 2-[β-(4-hydroxy-3-iodophenyl)ethylaminomethyl] tetralone (HEAT) were from Tocris Cookson. Puroxtone was a gift from Ferrosan. Yohimbine, SCH 23982, nisoxetine, and 2,5-dimethoxy-4-methylaminopipernidine were from Sigma-Aldrich. Radioligands were obtained from NEN Life Science Products and Amersham Pharmacia Biotech. [3H]Betaxolol was synthesized by Dr. J. Wursch (Hoffmann-La Roche AG). Mouse α1A, α1G, and α2D-adrenoceptor cDNAs were cloned and transfected into COS-7 cells by Dr. P. Malherbe (Hoffmann-La Roche AG).

**Cell Culture and Differentiation**—1C11 cells were grown and induced to differentiate (a) along the serotonergic pathway by the addition of 1 mM Bt2cAMP and 0.05% CCA in Dulbecco’s modified Eagle’s medium supplemented with fetal calf serum (10%) (8) or with 5-HT-depleted fetal calf serum (10%). 1C11**/NE cells were seeded at 4 × 10^4 cells cm^-2.

**Determination of Cellular Content of Bioamines, Enzymatic Activities, Uptake Experiments**—Bioamine contents, metabolites, related enzymatic activities, and uptakes were measured as described (8). Mouse brain synaptosomes were obtained according to O’Reilly and Reith (13).

**Radioligand Bindings and Related Experiments**—Binding experiments were performed on cell membranes as described (11). 1C11:**/NE cells were screened at days 2 and 4 of differentiation, 1C11**/NE cells were screened at days 4, 8, and 12. Specific radiolabeled ligands and their concentrations were chosen according to Alexander et al. (14) for adrenergic receptors (α1, α2, β), dopaminergic (D1, D2), and serotonergic receptors. The specific binding was defined as the one inhibited by 1 μM homologous unlabeled ligands. We favored use of the cold form of the radioligand to detect as many binding sites as possible, including non-specific ones, and thereafter to characterize them. The mean threshold for detection was 20 binding sites per cell. Determination of endogenous inositol 1,4,5-trisphosphate (IP3) was performed as described (11).

**Immunocytochemistry**—Indirect immunofluorescence experiments were made using rabbit polyclonal antibodies against L-CAM (a gift of Dr. N. Peyrissas), nestin (a gift of Dr. U. Lendhal), 5-HT (Eurodiagnostica), γ-ensolase (a gift of Dr. L. Legault), and NE (Roche Molecular Biochemicals); goat polyclonal antibodies against Notch-1 and Jagged-1 (Santa Cruz Biotechnology); mouse anti-NFL and rat anti-NCAM monoclonal antibodies (Valbiotech).

**RESULTS**

**1C11 Behaves as a Neuroectodermal Progenitor Inducible toward a Serotonergic or a Catecholaminergic Differentiation Program**—Under long term standard culture conditions, the 1C11 clone maintained an immature phenotype with an epithelial morphology (Fig. 1A); it expressed L-CAM (Fig. 1B), a marker of epithelial cells, as well as nestin (Fig. 1C), a marker of embryonic neuroepithelial cells (15). 1C11 cells were also positively stained by specific antibodies against Notch-1 and its ligand Jagged-1 (Fig. 1 D and E), two key regulatory molecules involved in neurogenesis (16). Conversely, the 1C11 progenitor lacked neuronal functions (Table I) and never spontaneously entered a differentiation program.

The 1C11 cell line may be recruited toward neuronal differentiation by Bt2cAMP, which most likely mimics the signaling of an embryonic factor. After the addition of this inducer and Me2SO, cells adopted a neural-like morphology (Fig. 1F). They kept dividing, albeit at a slower rate than 1C11 precursor cells (generation time 30 h versus 20 h). Bipolar extensions were already visible after 24 h of treatment, and neuron-associated markers such as N-CAM, synaptophysin (8), γ-ensolase (Fig. 1H), and neurofilament (Fig. 1I) were expressed. Four days after the addition of the inducers, nearly 100% of the cells could be positively stained by anti-5-HT antibodies (Fig. 1G). The catecholaminergic cells, now referred to as 1C11**/5HT cells, displayed a complete serotonergic metabolism with the ability to synthesize, store, and catabolize 5-HT, as inferred by the measurements of tryptophan hydroxylase activity, 5-HT content, and monoamine oxidase-B activity, respectively (Table I).

The addition of Me2SO, in combination with Bt2cAMP and CCA, inhibited the serotonergic program and instead promoted the catecholaminergic differentiation of 1C11 precursor cells. The addition of Me2SO alone (1 to 3%) in the culture medium induced neither a morphological change of 1C11 cells nor the expression of neuronal markers (data not shown). The effect of Me2SO concentration (in synergy with Bt2cAMP) on the onset of the catecholaminergic pathway is shown on Fig. 2A. A 2% concentration was selected to trigger the catecholaminergic differentiation of 1C11 cells under standard culture conditions, since a toxic effect was observed if the concentration exceeded 3%.

Within 4 days, Bt2cAMP/CCA/Me2SO-treated 1C11 cells, now referred to as 1C11**/NE cells, acquired a morphology with short, widely branching neurites, markedly distinct from the morphology of 1C11**/5HT cells (Fig. 1J). However, similarly to 1C11**/5HT cells, 1C11**/NE cells expressed N-CAM (Fig. 1L), neurofilament (Fig. 1M), and synaptophysin (not shown). The growth rate of 1C11**/NE cells was comparable with that of 1C11**/5HT cells. Catecholaminergic differentiation occurred in a reproducible and synchronous manner, with almost 100% of the cells being positively stained by anti-NE antibodies at day 4 (Fig. 1K). Tyrosine hydroxylase (TH) activity, the key enzyme of catecholamine biosynthesis, as well as DOPA, dopamine (DA), and NE contents became measurable (Table I). Like noradrenergic neurons (17), 1C11**/NE cells had a monoamine oxidase A activity (Table I) but no phenylethanolamine-N-methyltransferase activity (epinephrine synthesis) (Table I). Thus, we may conclude that the noradrenergic choice has been selective, with none of the serotonergic-related functions being expressed along the catecholaminergic pathway (Table I).

**The Catecholaminergic Program Induces the Onset of a Complete Catecholaminergic Phenotype—1C11**/NE cells went through a series of distinct maturation stages during their differentiation kinetics. Two days after the inducers were added, 1C11**/NE cells acquired a weak TH activity (Table IIA). However, NE content could not be measured until day 4, when the cells had developed a complete catecholaminergic metabolism (Table IIA). At this stage, the cells are not fully differentiated, as reflected in particular by the absence of any detectable NE or DA uptake (<1.5 pmol/mg of protein/min).

**In vivo**, during the differentiation of noradrenergic neurons, TH activity occurs before NE uptake (18). Therefore, we expected that the induction of the noradrenergic transporter (NET) may be part of the intrinsic catecholaminergic program of 1C11 cells. Accordingly, an active NE uptake could be measured 12 days after the inducers were added, provided that cells had been seeded at a lower density (10^6 cells/cm^2) and left to grow without replating to preserve neuronal polarity. This NE transport was blocked at 4 °C and responded to ouabain, an inhibitor of the Na^+–K^+–ATPase energy source. It was stationary after 1 min at 37 °C, and the apparent Km of NE (430 ± 20 nM) was close to that reported for HEla cells stably transfected with the human NET cDNA (19). The corresponding V_max value
was estimated at 12.5 ± 3.4 pmol/mg of protein/min. In parallel, [3H]nisoxetine, a selective inhibitor of NE uptake, bound intact cells with an apparent $K_d$ of 15.4 ± 1.2 nM and an apparent $B_{max}$ of 7.4 ± 0.5 fmol/mg of protein (220 binding sites/single cell). Finally, we performed competition experiments for [3H]nisoxetine binding to the NET present at the surface of 1C11**/NE catecholaminergic cells and to the NET of mouse brain synaptosomes. As shown in Fig. 2B, a significant correlation was found between the two pharmacological profiles.

Interestingly, between day 4 and day 12 of the catecholaminergic program, the levels of DOPA, DA, and NE in 1C11**/NE cells did not vary significantly (Table IIA). Thus, we may conclude that although the catecholaminergic metabolism is fully acquired after only 4 days of induction, cell differentiation takes 8 more days before the expression of a functional NE transport and, thereby, of a complete catecholaminergic phenotype.

**Fig. 1.** Immunocytochemical characterization of the 1C11 neuroepithelial precursor cell and its resulting 1C11**/5HT serotoninergic and 1C11**/NE catecholaminergic progeny. **A–E,** phase picture (A) and homogenous staining of 1C11 epithelial-like cells with antibodies to L-CAM (B), nestin (C), Notch-1 (D), and Jagged-1 (E). **F–I,** phase picture (F) and homogenous staining of 1C11**/5HT serotoninergic cells at day 4 with antibodies against 5-HT (G), γ-galactosidase (H), and NFL (I). **J–M,** phase picture (J) and homogenous staining of 1C11**/NE catecholaminergic cells at day 4 with antibodies against NE (K), N-CAM (L), and NFL (M). Scale bar = 25 μm.
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Table I
Serotonergic or catecholaminergic cell fate of 1C11 progenitor cells

|                | 1C11 | 1C11+/5HTd4 | 1C11+/5HTd4 |
|----------------|------|-------------|-------------|
| TPH (pmol/mg protein/30 min) | <0.5 | <0.5        | <0.5        |
| 5-HTP (pmol/mg protein)       | <0.5 | 21 ± 4      | 4 ± 0.5     |
| AADC (pmol/mg protein/30 min) | 5 ± 1 | 30 ± 4      | 23 ± 5      |
| 5-HT (pmol/mg protein)        | 0.21 | 26 ± 15     | <0.1        |
| MAO-B (pmol/mg protein/30 min)| <0.3 | 28 ± 6      | <0.3        |
| 5-HIAA (pmol/mg protein)      | <0.6 | 12 ± 5      | <0.6        |
| TH (pmol/mg protein/30 min)   | <0.5 | 0.5         | 65 ± 16     |
| DOPA (pmol/mg protein)        | <0.8 | 0.8         | 35 ± 8      |
| DA (pmol/mg protein)          | 0.1  | 0.1         | 266 ± 21    |
| DBH (pmol/mg protein/30 min)  | <0.7 | <0.7        | 23 ± 5      |
| PNX (pmol/mg protein/30 min)  | 0.3  | 0.3         | <0.2        |
| NE (pmol/mg protein)          | <0.1 | >0.1        | 74 ± 12     |
| MAO-A (pmol/mg protein)       | <0.4 | >0.4        | 14 ± 4      |

Binding experiments with [3H]GR12935 (<0.04 pmol/mg of protein/min from day 0 to day 12) excluded the presence of the DA transporter. Furthermore, 5-HT transporter molecules, assessed by [3H]-paroxetine binding (<0.03 pmol/mg prot/min), were never detectable during 1C11+/5HTd4 differentiation. Consequently, the choice between the serotonergic and the noradrenergic phenotypes was also reflected by the selective induction of the key enzyme tryptophan hydroxylase (TPH) and for NE (aromatic amino acid decarboxylase (AADC) and the key enzyme tyrosine hydroxylase (TH), and the activities of the catalyzing enzymes monoamine oxidase (MAO) A and B, and (iii) neurotransmitter content. The lack of phenylethanolamine N-methyltransferase (PNMT) activity indicates that 1C11+/5HTd4 cells are not able to convert NE into epinephrine. 5-HTP, 5-hydroxytryptophan; 5-HIAA, 5-hydroxy indoleacetic acid; DBH, dopamine b-hydroxylase.

An α1D-Adrenoreceptor Is Selectively Induced at Day 8 of the Catecholaminergic Differentiation Program—The consistent, time-dependent acquisition of catecholaminergic functions along the catecholaminergic differentiation pathway prompted us to investigate into the presence of neurotransmitter receptors on 1C11+/5HTd4 cell membranes. With binding experiments, the presence of 5-HT or DA receptor subtypes at any time in the absence of any agonist was verified that the number of HEAT binding sites and the pharmacologic profile of the α1D receptor did not change between isolated nerve cells.

Results emphasize (i) the onset at day 8 of a functional α1D-adrenoreceptor along the catecholaminergic pathway and (ii) its induction of a constitutive activity for the coupling of this receptor to PLCβ at day 10 of the differentiation program.

The α1D-Adrenoreceptor Actively Participates to the Acquisition of a Functional NE Transport in 1C11+/5HTd4 Catecholaminergic Cells—The onset of an α1D-adrenoreceptor before the completion of the differentiation program prompted us to examine whether catecholaminergic functions could be regulated by NE. The experiments were performed with cells left to differentiate in a dialyzed culture medium, where the external concentrations of NE, DA, and 5-HT were less than 2 nM. First, the α1D-adrenoreceptor was stimulated by the addition of 100 nM NE at day 8. As shown before, an increase in IP3 production was observed under these conditions. The parameters of NE metabolism and transport were measured 4 days later (day 12). They remained unchanged (Table IIB) as compared with the results obtained in standard culture conditions. The addition of NE at day 10 had no effect either on NE metabolism and transport, as measured at day 12.

In another experiment, the coupling of the receptor to PLCβ was inhibited by 10 nM full antagonist HEAT (Table IIB). Although the catecholaminergic metabolism was insensitive to this treatment (Table IIB), nisoxetine binding and NE transport, normally detected at day 12, became no more measurable (Table IIB). Strikingly, however, if HEAT was added at day 10, NE transport as well as NE metabolism remained intact at day 12 (Table IIB).

The above experiment suggested that the occurrence of catecholaminergic functions during the 1C11 serotonergic differentiation step had occurred between day 8 and day 10. Such a step may correspond to the acquisition of a constitutive activity for PLCβ coupling by the α1D-adrenoreceptor at day 10, as described in the previous paragraph. To assess this idea, we followed the PLCβ coupling as a function of HEAT addition. As shown in Table IIC, the addition of 10 nM HEAT at day 8 abolished the basal level of IP3 production, which we normally observed from day 10. If HEAT was added at day 10 instead of day 8, the constitutive activity of the receptor was maintained (Table IIC). These experiments clearly indicate that day 10 constitutes a critical commitment step during implementation of the catecholaminergic program by which neither the constitutive activity of the adrenoreceptor nor the induction of NE transport may be impaired by antagonist treatment. We can therefore suggest that the onset of NE transport is related to the α1D-adrenoreceptor basal signaling activity in 1C11+/5HTd4 cells.

External 5-HT Down-regulates All Serotonergic Functions Induced along the Serotonergic Differentiation Program—The onset of serotonergic functions during the 1C11 serotonergic program follows a well-defined kinetic pattern as well (Table III). Since 5-HT2B and 5-HT1D receptors were present in 1C11+/5HTd4 cells as early as day 2 of the serotonergic differentiation program, we wondered whether these receptors could modulate the serotonergic functions, similarly to what we observed for the catecholaminergic pathway. Because standard 10% FCS-supplemented medium contains 0.5 to 1 μM 5-HT, the culture medium was dialyzed, and the 5-HT level was reduced to less than 1 nM. The parameters for serotonergic differentiation were assessed for 1C11+/5HTd4 cells grown in 5-HT-depleted medium. Under these new culture conditions, the time sequence of acquisition of serotonergic functions remained un-
changed. However, at day 4, 5-HT cellular content \( (x \approx 2.8) \), tryptophan hydroxylase activity \( (x \approx 9.3) \), and the apparent \( V_{\text{max}} \) of 5-HT transport \( (x \approx 7.7) \) were higher than the values obtained with 1C11\(^{se/NE}\) cells grown in 10% FCS medium (Table III). These phenotypic changes specifically resulted from 5-HT starvation. Indeed, the values again became very close to those obtained under standard culture conditions if 5-HT concentration in the dialyzed medium was increased to 0.5 \( \mu \text{M} \) at day 0 of the serotonergic program (Table III). This experiment interestingly indicates a negative feedback of extracellular 5-HT on the extent of 5-HT synthesis and storage as well as on the 5-HT transport system itself.

**DISCUSSION**

The 1C11 cell line was already shown to produce serotonergic cells upon differentiation (8). In the present study, we show that the 1C11 cell actually is a bipotential neuroectodermal progenitor able to also convert into fully functional catecholaminergic cells. This report also emphasizes that the receptors selectively express in COS cells, a mouse neuroectodermal cell line (8), are able to also convert into fully functional catecholaminergic cells upon differentiation (8). In the present study, we show that the 1C11 cell actually is a bipotential neuroectodermal progenitor able to also convert into fully functional catecholaminergic cells. This report also emphasizes that the receptors selectively express in COS cells, a mouse neuroectodermal cell line (8), are able to also convert into fully functional catecholaminergic cells upon differentiation (8).

The 1C11 cell line initially resulted from the direct induction in vitro of F9-PK4 multipotential cells by retinoic acid and Bt\(2\)-cAMP (7). Despite the lack of a proper embryonal environment, 1C11 cells appear to have been irreversibly committed toward a neural fate. Neuroectodermal cell fate decision is influenced by a variety of signals, either endogenous, such as developmentally expressed bioamines (20) and neurotrophins, or exogenous, originating from the somites and the notochord (21). Among the complex network of pathways that dictates the developmental choice of neural progenitors, the Notch signaling pathway is of critical importance (16). The combined expression of Notch-1 and Jagged-1 in 1C11 cells and the homogenous immunostaining of the cell nuclei with Notch-1 antibodies suggest that Notch-ligand interactions may take part in determining the fate of 1C11 cells. Finally, the expression of the early neuroectodermal marker nestin together with the lack of expression of neuronal functions indicate that the 1C11 clone may correspond to a neuroepithelial precursor.

**1C11 Catecholaminergic Differentiation**—The PC12 cell line and the MAH sympathoadrenal progenitor (22) have already enabled the study of several features of the catecholaminergic differentiation. In particular, PC12 cells express a phenotype of transformed chromaffin cells and respond to nerve growth factor by adopting a neuron-like fate, with a switch from the adrenergic to a cholinergic phenotype (23). The 1C11 clone provides another, yet different, model for catecholaminergic differentiation. In contrast to the adrenergic cells of the peripheral nervous system or adrenal medulla, 1C11\(^{se/NE}\) cells lack phenylethanolamine-N-methyltransferase enzymatic activity.

The 1C11 catecholaminergic program was triggered by the
synergistic effect of Bt-cAMP and Me_SSO, whereas the serotonergic program was obtained with Bt-cAMP alone. Me_SSO therefore acts as a dominant epigenetic signal. Such an effect of Me_SSO can be related to that described in the case of Friend erythroleukemia cells (24) and of NIE-115 neuroblastoma cells (25), where Me_SSO confers a neuronal morphology.

The catecholaminergic program is restricted to the induction of (i) the catecholamine metabolism, (ii) an adrenoreceptor, and (iii) NE uptake. Several features of differentiation are reminiscent of the in vitro situation. Similarly to the noradrenergic neurons of the brainstem (26), 1C11*NE cells have more robust but shorter neuritic processes than 1C11*5HT cells. As in vitro (18), the synthesis of catecholamines occurs before NE uptake. Eventually, like noradrenergic neurons, 1C11*NE noradrenergic cells selectively acquire a functional α1D-adrenoreceptor at day 8 of differentiation. Although it appears not to be implied in the catecholaminergic metabolism, this receptor is involved in the completion of the phenotype. From our various observations, we may draw a link between the induction of a constitutive activity for the receptor at day 10 and the NET regulation. Indeed, inactivation of the receptor at day 8 impairs (i) NE transport and nisoxetine binding at day 12 and (ii) the onset of a constitutive PLC coupling to the receptor at day 10. None of these effects are observed if the antagonist treatment is applied at day 10.

Since these observations could be gained in culture medium deprived of exogenous catecholamines, we may propose that the acquisition by the receptor of its constitutive signaling activity is sustained by NE secreted by the cell itself. It is likely that 1C11*NE cells produce NE as soon as day 4, when NE metabolism is implemented. A possible hypothesis is that small granular pulses of catecholamines, the existence of which we are currently testing by fast scan cyclic voltammetry, may account for NE release. Whatever the origin of the release, endogenous NE and the ensuing autocrine stimulation of the α1D-adrenoreceptor appear sufficient to allow the onset of a functional NE transport. Indeed, the kinetics of induction and the parameters of NE transport at day 12 are not improved by prior addition of NE in the culture medium at day 8 or 10.

The role of α1D receptors in vitro is unclear. The present discovery that an adrenoreceptor may act as an autoreceptor

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**Table II**

Kinetics of acquisition of catecholaminergic functions by 1C11*NE catecholaminergic cells and involvement of the α1D-adrenoreceptor in the onset of the NET

| A | 1C11*5HTd2 | 1C11*5HTd4 | 1C11*5HTd8 | 1C11*5HTd12 |
|---|-----------|------------|------------|-------------|
| TH (pmol/mg protein/30 min) | 18 ± 4 | 65 ± 16 | 87 ± 13 | 90 ± 10 |
| DOPA (pmol/mg protein) | 10 ± 3 | 35 ± 8 | 55 ± 9 | 52 ± 10 |
| DA (pmol/mg protein) | 60 ± 18 | 266 ± 21 | 273 ± 14 | 267 ± 12 |
| DBH (pmol/mg protein/30 min) | 1.8 ± 0.2 | 23 ± 5 | 23 ± 3 | 22 ± 5 |
| NE (pmol/mg protein) | <0.1 | 74 ± 12 | 76 ± 12 | 79 ± 10 |

**Table III**

Kinetics of acquisition of serotonergic functions by 1C11*5HT cells and 5-HT-induced down-regulation of the serotonergic phenotype

| B | 1C11*5HTd12 (1) | 1C11*5HTd12 (2) | 1C11*5HTd12 (3) | 1C11*5HTd12 (4) |
|---|----------------|----------------|----------------|----------------|
| NE uptake | TH (pmol/mg protein/30 min) | 90 ± 10 | 87 ± 20 | 86 ± 12 | 74 ± 17 |
| K<sub>0</sub> (nM) | 434 ± 21 | 455 ± 17 | No detectable uptake | 456 ± 20 |
| V<sub>max</sub> (pmol/mg protein/min) | 12.5 ± 3.4 | 13.1 ± 5.4 | No detectable binding | 12.3 ± 2.6 |
| Nisoxetine binding | K<sub>x</sub> (nM) | 15.4 ± 1.2 | 16.1 ± 1.3 | No detectable binding | 14.2 ± 1.6 |
| B<sub>max</sub> (pmol/mg protein) | 0.74 ± 0.05 | 0.75 ± 0.14 | 0.71 ± 0.08 |

| C IP3 (10<sup>-18</sup> mol/cell) | Day 8 | Day 10 | Day 12 |
|---|---|---|---|
| 0.5 | 5.5 | 7.3 |

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A, NE-related functions were measured at various steps (days 2, 4, 8, and 12) of the 1C11*NE catecholaminergic differentiation program. DBH, dopamine β-hydroxylase. B, the parameters for NE metabolism and transport were measured at day 12 of differentiation: in 1C11*NE cells grown in a dialyzed medium (1C11*NE d12, column 1), after an activation of the α1D-adrenoreceptor by 100 nM NE at day 8 (column 2), or after blockade of the receptor by 10 nM HEAT at day 8 (column 3) and day 10 (column 4). C, the basal level of IP<sub>3</sub> production in 1C11*NE cells grown under standard conditions was measured at days 8, 10, and 12 of the catecholaminergic differentiation program (column 1) or after inhibition of the α1D-adrenoreceptor by addition of 10 nM antagonist HEAT at day 8 (column 2) or day 10 (column 3).

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**Table II**

Kinetics of acquisition of catecholaminergic functions by 1C11*NE catecholaminergic cells and involvement of the α<sub>1D</sub>-adrenoreceptor in the onset of the NET
interfering with the onset of the NE transporter is unprecedented, yet NE binding to α-adrenoreceptors has already been suggested to be involved in the differentiation of amphibian embryonic neurons (28).

**1C11 Cells and Serotonergic Differentiation**—The availability of cellular models exhibiting a complete serotonergic phenotype, i.e., 5-HT metabolism, storage, uptake, and receptors, is of general interest in view of the involvement of serotonergic neurons in many behavioral and homeostatic processes. The few cell lines meeting these criteria include the thyroid parafollicular cell system (29) and the embryonic raphé-derived RN46A cell line (30). Despite their ability to undergo differentiation after induction, these systems already display functional serotonergic properties in the undifferentiated state. In contrast, serotonergic-related functions cannot be detected in the 1C11 progenitor.

The time schedule enabling the acquisition of serotonergic functions by 1C11+/5HT cells is consistent with in vivo observations. The phenotype reaches completion within 4 days, and the expression of a functional 5-HT transport takes place only after the induction of tryptophan hydroxylase activity (Table III). In the rat, the ontogeny of central serotonergic neurons, originating in the raphe nuclei, also occurs within a window of 4 days, between day 11 and day 15 of embryogenesis. At day 13, neurotransmitter synthesis becomes measurable and monoamine transporters cannot be detected before day 15 (31).

In the present study, the intensities of 5-HT synthesis, storage, and uptake are shown to be down-regulated by 5-HT itself through the 5-HT2B and/or 5-HT1D receptors already present on the cells at day 2. The cells become responsive to external 5-HT at day 2 when the onset of 5-HT2B and 5-HT1D receptors occurs. This day therefore appears to be a critical commitment step within the 1C11 serotonergic program. The two receptors are still functional at day 4, when 5-HT2A receptors become expressed. Likely all these receptors can mediate the effect of 5-HT in the coordination of the serotonergic functions underlying a complete phenotype of the cells. In fact, 5-HT1D receptors have already been proposed to behave as autoreceptors capable of modulating serotonergic neurotransmission (32).

Our unpublished data suggest that the 5-HT2B receptor may also act as an autoreceptor, as it controls the overall 5-HT transport system of 1C11+/5HT cells.

**Concluding Remarks**—The 1C11-inducible cell line provides an example of how the autonomous genetic program of a precursor cell can lead to the progressive acquisition of neuronal- and neurotransmitter-related functions up to a complete and autoregulated phenotype. Although it has never been clearly established that serotonergic and noradrenergic cells may share a common precursor in the course of embryonic development, the dual bioaminergic fate of 1C11 cells is in line with two very recent reports (5, 33), which suggest that central dopaminergic and serotonergic neurons indeed arise from a common progenitor. The consistency of the 1C11 differentiation programs lies on (i) the 100% phenotypic conversion, (ii) the implementation of all functions specific to serotonergic or noradrenergic neurons, in a mutually exclusive manner, (iii) the temporal orders of onset of neurotransmitter-related features that are similar to those observed in vivo, (iv) the selective induction of bioaminergic receptors along either pathway, sustaining the autoregulation of the corresponding phenotype. Hopefully the 1C11 system may, in view of these key properties, help gain insight into the cellular and molecular mechanisms involved in the modulation of neuronal differentiation by neurotransmitters.

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