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A CNS Catecholaminergic Cell Line Expresses Voltage-gated Currents

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Abstract. CATH.a is a central nervous system (CNS) catecholaminergic cell line derived from a transgenic mouse carrying the SV40 T antigen oncogene under the transcriptional control of regulatory elements from the rat tyrosine hydroxylase gene (Suri et al., 1993). CATH.a cells express several differentiated neuronal characteristics including medium and light chain neurofilament proteins, synaptophysin, tyrosine hydroxylase, and dopamine β-hydroxylase; they synthesize dopamine and norepinephrine. Conversely, they do not express glial-specific fibrillary acidic protein.

To establish definitively that CATH.a cells are of neuronal origin, we characterized the repertoire of voltage-gated inward currents expressed by CATH.a cells. Such inward currents are necessary for neuronal excitability. We report that all CATH.a cells possess a tetrodotoxin-sensitive sodium current (peak amplitude = 590 ± 319 pA) and 68% possess a high voltage-activated calcium current (peak amplitude = 175 ± 67 pA). Pharmacological analyses suggest that individual cells express varying levels of L- and N-type calcium current, but no P-type current. In addition, in 55% of the cells with a calcium current, about a half of this current is resistant to selective antagonists for L- and N-type currents, suggesting that another calcium current exists in these CATH.a cells which is not L-, N-, or P-type. The heterogeneous pattern of current detected persisted in several CATH.a subclones, suggesting that factors other than genetic variability influence current expression.

The demonstration that CATH.a cells express these currents indicates that they have excitable membrane properties characteristic of neurons. Although many peripheral nervous system (PNS) cell lines exist, very few CNS cell lines with differentiated neuronal properties exist. Since the CATH.a cells can be grown continuously in large amounts, they may be useful for purifying, characterizing, and/or cloning various neuronal-specific molecules and thereby may add to our understanding of CNS catecholaminergic neurons.

Key words: Catecholaminergic — Cell line — Neuroblastoma — Voltage clamp — Sodium current — Calcium current

Immortalized cell lines with differentiated neuronal properties offer unique advantages for studying neuronal function. They provide a homogeneous source of a single cell type which can be grown indefinitely in large quantities, allowing the characterization and purification of neuronal-specific molecules. Central nervous system (CNS) cell lines are especially valuable because of the difficulty in preparing pure CNS cultures of defined neuronal subpopulations in significant quantities.

Several catecholaminergic cell lines exist. All, except the CATH.a line (*see below*) are partially derived from the peripheral nervous system (PNS). Many sympathetic neuroblastomas exist, in addition to lines from adrenal medullary tumors. The most widely used catecholaminergic cell line is the neural crest-derived PC12 pheochromocytoma line which arose from a rat adrenal chromaffin tumor (Greene & Tischler, 1976).

While the neuroblastoma and pheochromocytoma lines are purely PNS in origin, several somatic hybrid lines (MN9D and the MES23.5 lines) have been generated by fusion of a PNS neuroblastoma and a primary embryonic CNS catecholaminergic neuron (Choi et al., 1991; Crawford et al., 1992). Although the hybrid lines express differentiated properties, it is difficult to deter-

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mine to what degree PNS characteristics dominate or modify CNS-specific traits as chromosomes from both CNS and PNS parents persist in the hybrids. This is of particular concern because genes can be activated that were previously silent in the parental cells (Minna, Yavelow & Coon, 1975; Mével-Ninio & Weiss, 1981).

To obtain a CNS cell line and avoid complications arising from biparental hybrids, tyrosine hydroxylase (TH)-expressing CNS tumors were induced in transgenic mice carrying the SV40T antigen oncogene under the transcriptional control of upstream elements from the rat TH gene. CATH.a was derived from such a brainstem tumor (Suri et al., 1993). CATH.a cells appear morphologically undifferentiated under normal culture conditions; only a small percentage possess short neurites. Nevertheless, they express some differentiated neuronal characteristics such as high levels of TH (the first and rate-limiting enzyme in catecholamine biosynthesis), synaptophysin, and neurofilament protein. They do not express glial fibrillary acidic protein which is characteristically expressed in glial cells. CATH.a cells accumulate and secrete high levels of dopamine and norepinephrine. These data suggest that CATH.a cells are immortalized derivatives of CNS catecholaminergic neurons (Suri et al., 1993).

To determine if CATH.a cells have membrane properties characteristic of neurons, we tested for the presence of voltage-gated inward currents using tight-seal, whole-cell recording (Hamill et al., 1981). The results indicate that CATH.a cells possess voltage-gated sodium and calcium currents. The sodium currents are tetrodotoxin-sensitive. The calcium currents are heterogeneous. Pharmacological results suggest the presence of L- and N-type calcium channels. However, in many cells, a significant fraction of the calcium current remains in the presence of selective antagonists for L-, N-, and P-type channels, suggesting that another calcium current (''resistant''- type) exists in these CATH.a cells which is not L-, N-, or P-type.

Because CATH.a cells exhibited heterogeneity with respect to the current type and size, independent subclones were isolated and recorded from to determine whether the CATH.a cells were truly clonal. Surprisingly, heterogeneity persisted within each subclone, suggesting that differences between individual cells are not primarily due to genetically different subpopulations within the parental CATH.a cultures or within each subclone.

This study and previous work (Suri et al., 1993) demonstrate that CATH.a cells express a neuronal phenotype. Since the CATH.a cells can be grown continuously in large amounts, they may be useful for purifying, characterizing, and/or cloning various neuronal-specific molecules such as a ''resistant''-type calcium channel. In addition, the CATH.a cells have been shown to be useful for investigating induction of TH transcription in response to membrane depolarization (*unpublished data*). Future work will involve examining the mechanism of depolarization-induced TH transcription and possibly isolating the transcription factor(s) involved.

Materials and Methods

Cell Culture

Cells were grown at 37°C and in 5% CO_2 on tissue culture plastic (Falcon, Becton Dickinson, Lincoln Park, NJ) in RPMI 1640 media supplemented with 8% horse serum (Gibco, Grand Island, NY), 4% fetal bovine serum (Hyclone, Logan, UT), and 1% penicillin-streptomycin (100% stocks were 10,000 U/ml penicillin G and 10,000 μg/ml streptomycin sulfate; Gibco). 24–48 hr before recording, cells were passaged using 0.25% trypsin without EDTA (Gibco) to disrupt cell clumps; the cells were replated on 35 mm Falcon tissue culture plates at low density such that isolated cells were easily obtained for recording.

CATH.a Subclones

The CATH.a cells were subcloned twice in 96-well microtiter tissue culture plates (Falcon). Clones (colonies derived from a single cell) were identified by visual inspection and selected. Four subclones, C12C1, A12A2, A5H8, and A5G11, were obtained. A5H8 and A5G11 arose from the same parental subclone in the first cloning cycle, while C12C1 and A12A2 were independent isolates.

Electrophysiology

CATH.a cells (or the various subclones) on tissue culture plates were rinsed once with phosphate buffered saline and once with the appropriate external bath solution. The cells were then placed in the external bath solution used for whole-cell recording. Standard tight-seal, whole-cell recordings were performed at room temperature with a List EPC-7 patch-clamp amplifier. Current traces were digitized at 5–10 kHz. These were saved and analyzed on an Atari Mega 4 ST computer using data acquisition and analysis software from Instrutech (Elmont, NY). Displayed records were not filtered further. A standard P/4 protocol starting from a holding potential of −100 mV was used to subtract linear components of capacitive and leak current from all traces. Although the cells varied in size, no correlation was observed between current amplitude and membrane surface area (proportional to cell capacitance). We, therefore, have reported most data in terms of current amplitudes (pA). Voltage steps were applied to the test cell every 3 sec. Fisherbrand (Pittsburgh, PA) glass microhematocrit capillary tubes (No. 02-668-68) were used to make patch pipettes. These were filled with the appropriate internal solution (*see below*). Pipette resistances prior to forming high-resistance seals ranged from 1.75 to 4.0 megaohms.

Solutions

Solutions used for whole-cell recordings are described in Table 1. For a general characterization of voltage-gated currents present in CATH.a cells and the CATH.a subclones, we used External solution #1

Table 1. Whole cell recording solutions

External solutions	NaCl	KCl	$CaCl_2$	$MgCl_2$	Glucose	NaOH	HEPES	Other
1	121	5	3	1	10		10	
2	121	5	3	1	10		10	25 mM TEA-Cl 50 μM $CdCl_2$
3	93		3	0.8	5	12.5	25	50 mM TEA-Cl 100 μM $CdCl_2$
4	118		3	0.8	5	12.5	25	25 mM TEA-Cl 100 μM $CdCl_2$
5	107.5		10	0.8	5	12.5	25	250 nM TTX 25 mM TEA-Cl

Puffer solutions	NaCl	$CaCl_2$	$MgCl_2$	Glucose	NaOH	HEPES	Other
1		3	0.8	5	12.5	25	93 mM NMDG 50 mM TEA-Cl 100 μM $CdCl_2$
2	118	3	0.8	5	12.5	25	250 nM TTX 25 mM TEA-Cl 100 μM $CdCl_2$
3	107.5	10	0.8	5	12.5	25	250 nM TTX 25 mM TEA-Cl
4	107.5	0.1	10.7	5	12.5	25	250 nM TTX 25 mM TEA-Cl

Internal solutions	NaCl	Potassium aspartate	$MgCl_2$	Na_2ATP	MgATP	BAPTA	HEPES	CsCl
1	10	115	1.5	1.5		5	10	
2	10		1.5	1.5		5	10	140
3					5	5	10	150

Abbreviations are as follows: BAPTA (1,2-bis (2-aminophenoxy) ethane N,N,N′,N′-tetraacetic acid); HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); NMDG (N-methyl-D-glucamine); TEA-Cl (tetraethylammonium chloride); TTX (tetrodotoxin). All concentrations are given in mM except where indicated. All solutions are pH 7.4. In addition to the reagents presented in the table, Puffer solution #3 contained either 50 μM $CdCl_2$, 10 μM (+)-(S)-202-791, 5 μM nimodipine, 10 μM ω-conotoxin GVIA and 100 μg/ml BSA, or 200 nM ω-agatoxin-IVA and 100 μg/ml BSA.

and Internal solution #1. To examine sodium currents in isolation, we used External solutions #2, 3, and 4 and Puffer solutions #1 and 2, all containing cadmium and tetraethylammonium chloride (TEA-Cl) to block calcium and potassium currents, respectively and Internal solutions #2 and 3 containing cesium to block potassium currents. To examine calcium currents in isolation we used External solution #5 and Puffer solutions #3 and 4, all containing tetrodotoxin (TTX) and TEA-Cl to block sodium and potassium currents, respectively and Internal solution #3.

Chemicals

All drugs were stored as stock solutions at −20°C and then diluted in the appropriate external solution on the day of the experiment. Tetrodotoxin (TTX; Calbiochem, San Diego, CA) was stored as a stock solution of 10 mg/ml in distilled water; working concentration was 250 nM. (+)-(S)-202-791 and nimodipine were the generous gifts of Dr. Robert Hof, SANDOZ AG Preclinical Research (Basel, Switzerland) and Dr. Scriabine, Miles Pharmaceuticals, respectively. Stock solutions of these dihydropyridines were made at a concentration of 10 mM in 95% ethanol; working concentrations were 10 μM and 5 μM for (+)-(S)-202-791 and nimodipine, respectively. ω-conotoxin-GVIA (Bachem, Torrance, CA) was stored as a stock solution of 1 mM in distilled water; working concentration was 10 μM. ω-agatoxin-IVA (Peptides International, Louisville, KY) was stored as a stock solution of 100 μM in distilled water; working concentration was 100–200 nM. Drugs were applied via a puffer with a tip diameter of approximately 5 μM.

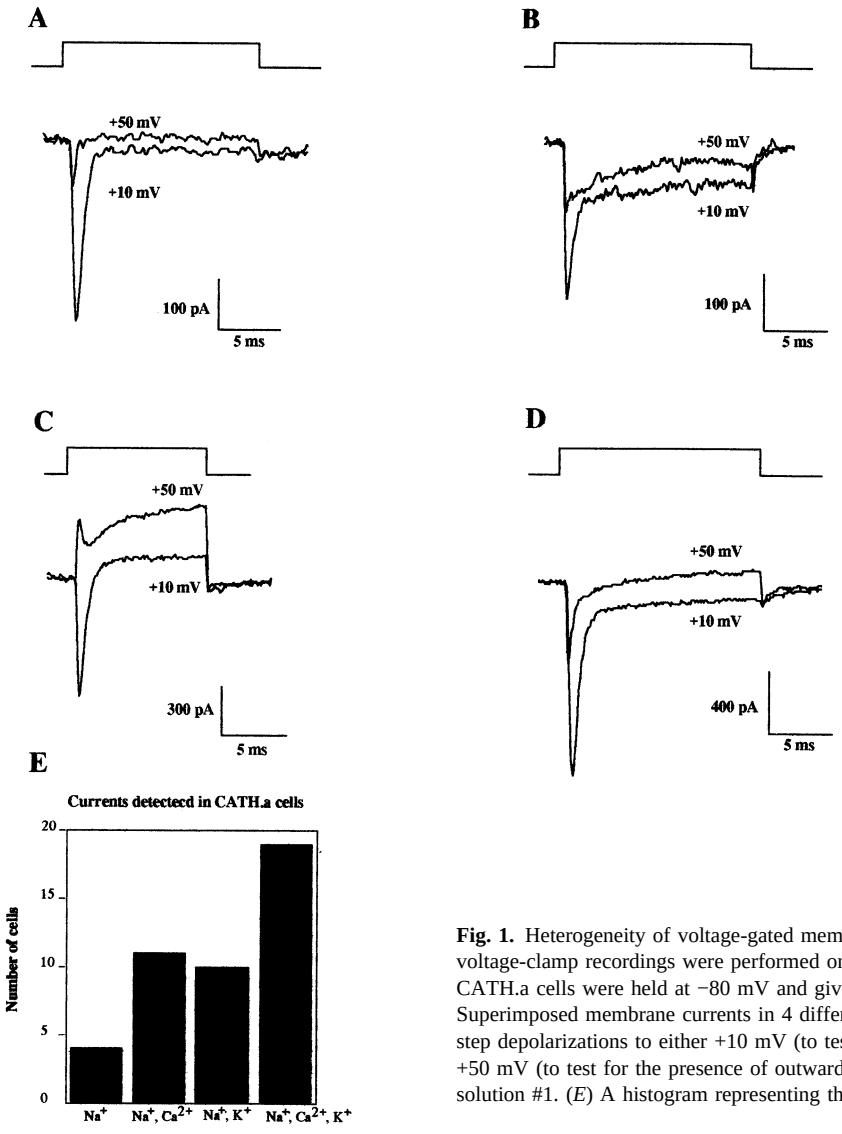

Fig. 1. Heterogeneity of voltage-gated membrane currents in CATH.a cells. Whole-cell voltage-clamp recordings were performed on a total of 44 cells from 6 different platings. CATH.a cells were held at −80 mV and given 10–20 msec test depolarizations. (*A–D*) Superimposed membrane currents in 4 different CATH.a cells measured in response to step depolarizations to either +10 mV (to test for the presence of inward currents) or to +50 mV (to test for the presence of outward currents). External solution #1; Internal solution #1. (*E*) A histogram representing the data from the 44 cells.

Results

General Properties of Voltage-gated Currents in CATH.a Cells

Whole-cell voltage-clamp recording revealed that the CATH.a cells express several biophysically distinct voltage-gated currents. We detected a rapidly inactivating inward current in all cells ($n = 44/44$), a long-lasting inward current in 68% of the cells ($n = 30/44$), and a delayed outward current in 66% of the cells ($n = 29/44$). The cells can be divided into four classes defined by the various combinations of currents detected: (i) a transient inward current alone (4/44; Fig. 1*A*), (ii) transient and long-lasting inward currents (11/44; Fig. 1*B*), (iii) transient inward and delayed outward currents (10/44; Fig. 1*C*), and (iv) transient inward, long-lasting inward, and delayed outward currents (19/44; Fig. 1*D*). A histogram summarizing these data is shown in Fig. 1*E*.

The transient inward current activated near −40 mV, reached its peak at −10 mV, and was similar to voltage-gated sodium currents observed in other excitable cells. The long-lasting inward current activated between −40 and −30 mV, peaked near +10 to +20 mV, and appeared similar to high voltage-activated calcium currents observed in other preparations. The delayed outward current was revealed with depolarizing pulses above 0 mV. It increased monotonically with increasing depolarization and appeared similar to some voltage-gated potassium currents. In cells where all three currents were detected, the long-lasting inward current was most easily observed at potentials between −10 and +10 mV. The delayed outward current masked the inward current at more depolarized potentials.

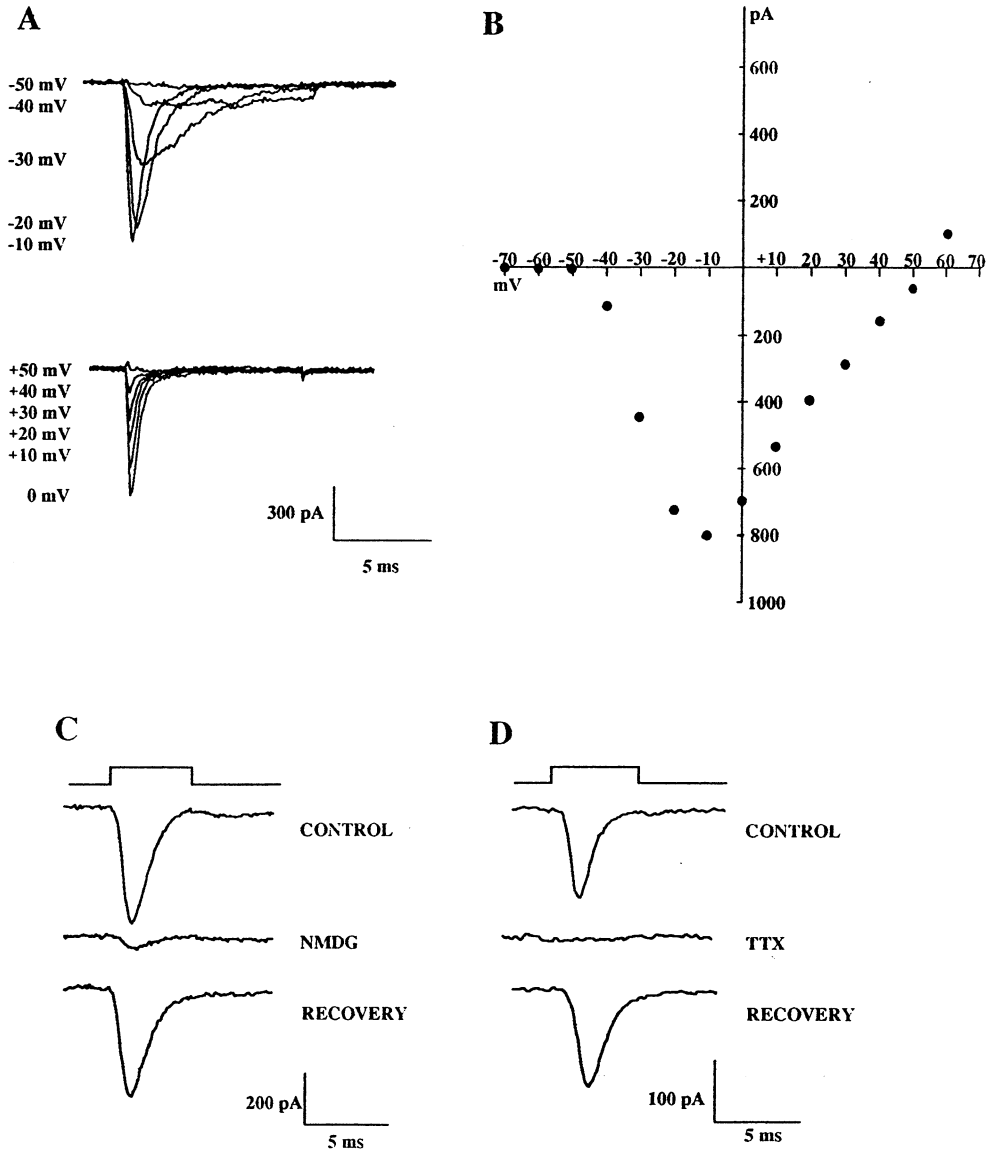

Fig. 2. The transient inward current is a sodium current. (*A*) Whole-cell voltage-clamp recordings were performed on 19 CATH.a cells from 3 different platings. Superimposed membrane currents from a CATH.a cell in response to 10-msec step depolarizations ranging from −50 to +50 mV (as marked) from a holding potential of −80 mV. (*B*) The *I-V* plot of the cell shown in *A*. External solution #2; Internal solution #2. (*C*) Whole-cell voltage-clamp recordings were performed on 4 CATH.a cells from 2 different platings. Cells were held at −80 mV and given 5-msec step depolarizations from −80 to 0 mV. Currents were recorded in control solution (CONTROL), a solution in which Na^+ was replaced with NMDG (NMDG), or following washout of the NMDG solution (RECOVERY). External solution #3; Puffer solution #1; Internal solution #3. (*D*) Whole-cell voltage-clamp recordings were performed on 4 CATH.a cells from 2 different platings. Cells were held at −80 mV and given 5-msec step depolarizations to 0 mV. Traces shown were taken before (CONTROL), during (TTX), or after washout (RECOVERY) of 250 nM TTX. External solution #4; Puffer solution #2; Internal solution #3.

The Rapidly Inactivating Inward Current is through Tetrodotoxin-Sensitive Sodium Channels

To examine the transient inward current in isolation, we blocked calcium and potassium currents (External solutions #2, 3, 4 and Internal solution #2, 3). Cadmium in the external solutions entirely eliminated the long-lasting inward current; external tetraethylammonium chloride (TEA-Cl) and internal cesium largely blocked outward currents, although they were occasionally detected at test potentials more positive than +60 mV.

The threshold for activation of this inward current was −40 ± 4 mV ($n = 19$; Fig. 2*A* and *B*). Peak current was reached at −10 ± 6 mV ($n = 19$; Fig. 2*A* and *B*).

Typically peak current amplitudes varied between 200 pA and 1000 pA (mean = 590 ± 319 pA; $n = 17$); however, in 2 other cells it approached 5 nA. Current densities ranged from 1.4–6.7 pA/μm^2. The current reversed at +55 ± 8 mV ($n = 19$; Fig. 2*A* and *B*). Rundown of the transient inward current was not observed in cells voltage-clamped for as long as 45 min.

Several observations support the contention that the transient inward current flows through voltage-dependent sodium channels. First, the reversal potential is near +55 mV, close to the calculated Nernst potential of +62 mV for sodium under our recording conditions. Second, the amplitude of the current greatly diminishes in a reversible manner at all test potentials from −80 to +80 mV when the NaCl in the external solution is replaced with N-methyl-D-glucamine (NMDG), which is impermeant through sodium channels (Fig. 2*C*). Finally, the current activates near −40 mV; at −10 mV, it activates within 0.7 msec and inactivates in an exponential manner ($\tau = 0.7$ msec). The reversal potential, sodium dependence, threshold potential, activation and inactivation kinetics are similar to those of voltage-dependent sodium currents characterized in other excitable cells (Fernandex, Fox & Krasne, 1984; Stühmer et al., 1987; Mandel et al., 1988; Barres, Chun & Corey, 1989).

Pharmacological experiments provided additional evidence for the presence of voltage-gated sodium channels in CATH.a cells. Puffer application of 250 nM tetrodotoxin (TTX), a specific and potent (K_d = 1–10 nM) sodium channel blocker, eliminated the transient inward current at all test potentials from −80 to +80 mV in CATH.a cells (Fig. 2*D*). This block was completely reversible following washout of TTX. Together these data argue strongly that the transient inward current in CATH.a cells is due to the activation of TTX-sensitive sodium channels.

The Long-lasting Inward Current is Due to the Activation of Calcium Channels

Sixty-eight percent of CATH.a cells exhibited a long-lasting inward current similar to high voltage-activated (HVA) calcium currents observed in other cells. To isolate this current, we blocked sodium and potassium currents (External solution #5, Internal solution #3). Only the long-lasting inward current was regularly observed.

The amplitude of the long-lasting inward current varied among different cells, ranging between 40–300 pA at its peak, and averaging 175 ± 67 pH ($n = 8$). Current densities ranged from 0.57–1.9 pA/μm^2. In contrast to previously characterized HVA calcium currents, rundown was insignificant in CATH.a cells voltage-clamped up to 20 min ($n = 10$); time points after 20 min were examined in 2 cells where rundown was not observed for as long as 45 min. However, similar to other HVA calcium currents, the current-voltage relationship (Fig. 3*A*,*B*) demonstrates that the long-lasting inward current activated between −40 and −30 mV, peaked between +10 to +20 mV, and reversed at +66 ± 6 mV ($n = 8$). The current was calcium dependent as it was blocked by cadmium, one of the several divalent cations known to effectively block voltage-gated calcium channels (Hagiwara & Byerly, 1981; 1983; Nachshen, 1984; Byerly, Chase & Stimers, 1985; Lansman, Hess & Tsien, 1986). Puffer application of 50 or 100 μM cadmium in the standard external solution reversibly blocked the inward current at all test potentials (−80 to +80 mV; Fig. 3*C*). Furthermore, the amplitude of the inward current was dependent on the external calcium concentration. When cells were switched from a standard high calcium external solution (10 mM) to a low calcium solution (0.1 mM), the long-lasting inward current became greatly diminished at all test potentials (−80 to +80 mV; Fig. 3*D* and *E*) and this effect was fully reversible. Together, these observations suggest that this current is generated through HVA calcium channels.

Pharmacological Characterization of Calcium Currents

Several types of HVA calcium current (L-, N-, and P-type) have been defined by their sensitivity to selective pharmacological agents: 1,4-dihydropyridines for L-type, ω-conotoxin-GVIA for N-type, and ω-agatoxin-IVA for P-type (Tsien et al., 1988; Bean, 1989; Hess, 1990; Catterall & Striessnig, 1992; Tsien, Ellinor & Horne, 1991; Llinás et al., 1992; Miller, 1992; Snutch & Reiner, 1992; Dunlap, Luebke & Turner, 1995). We utilized these agents to characterize calcium currents in CATH.a cells.

To test for the presence of L-type calcium currents, we employed both agonist and antagonist dihydropyridines (Hess, Lansman & Tsien, 1984; Nowycky, Fox & Tsien, 1985*a*; Hof et al., 1985; Kokubun et al., 1986; Fox, Nowycky & Tsien, 1987*a*,*b*; Hosey & Lazdunski, 1988; Plummer, Logothetis & Hess, 1989). 1,4-dihydropyridine agonists, such as (+)-(S)-202-791, prolong L-type channel open time and hence increase the whole-cell calcium current; they also shift the voltage dependence of activation causing the *I-V* relationship to shift to more hyperpolarizing potentials. In 15 out of 23 CATH.a cells, puffer application of 10 μM (+)-(S)-202-791 increased the calcium current by 102 ± 41% (at test potentials of −10 to 0 mV) and produced a hyperpolarizing shift in the *I-V* curve (Fig. 4*A* and *B*). The relatively slow voltage clamp provided by the high-resistance patch pipettes required for recording from the CATH.a cells prevented us from discriminating a change in tail current deactivation kinetics that is commonly observed with dihydropyridine agonists acting on L-type channels (Plummer et al., 1989).

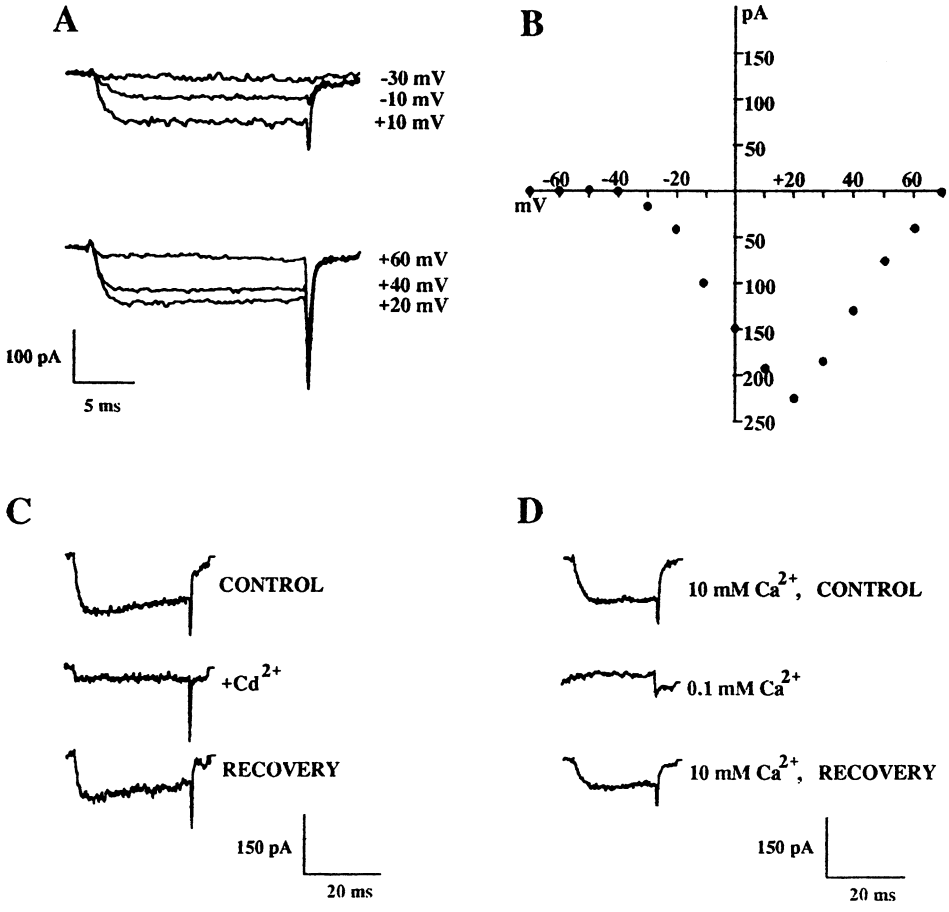

Fig. 3. The long-lasting inward current is a calcium current. (*A*) Whole-cell voltage-clamp recordings were performed on 8 CATH.a cells from 3 different platings. Superimposed membrane currents from a CATH.a cell in response to 20-msec step depolarizations ranging from −30 to +60 mV from a holding potential of −80 mV. (*B*) The *I-V* plot of the cell shown in *A*. External solution #5; Internal solution #3. (*C*) Whole-cell voltage-clamp recordings were performed on 7 CATH.a cells from 3 different platings. Cells were held at −80 mV and given 30-msec step depolarizations from −80 to +10 mV. Traces shown were taken before (CONTROL), during ($+Cd^{2+}$), and after washout of 50 μM Cd^{2+} (RECOVERY). External solution #5; Puffer solution #3; Internal solution #3. (*D*) Whole-cell voltage-clamp recordings were performed on 6 CATH.a cells from 4 different platings. Cells were held at −80 mV and given 25-msec step depolarizations from −80 to +10 mV. The traces were taken before (10 mM Ca^{2+}, CONTROL), during (0.1 mM Ca^{2+}), and after washout (10 mM Ca^{2+}, RECOVERY) of a solution containing 0.1 mM Ca^{2+}. External solution #5; Puffer solution #4; Internal solution #3. (*E*) Current-voltage relation of another CATH.a cell in 10 mM calcium external solution (filled circles) and after puffer application of a 0.1 mM calcium solution (open circles). External solution #5; Puffer solution #4; Internal solution #3.

Puffer application of 5 μM nimodipine, a dihydropyridine antagonist, reduced the calcium current evoked by step depolarizations from a holding potential of −80 mV by 42 ± 13% in 9 out of 10 CATH.a cells (Fig. 4*C*). Previous studies suggest that dihydropyridine antagonists work more effectively when cells are clamped at more positive holding potentials (Bean, 1984; Sanguinetti & Kass, 1984). However, we did not determine whether nimodipine blocked calcium currents more effectively when CATH.a cells were clamped at more positive holding potentials because control calcium currents evoked from holding potentials more positive than −50 mV were too small (<40 pA) to measure reliably. The (+)-(S)-202-791 and nimodipine effects on calcium currents suggest that part of the calcium current is carried through L-type calcium channels in 73% of the CATH.a cells (24/33 cells; combined results from nimodipine and (+)-S-202-791 experiments).

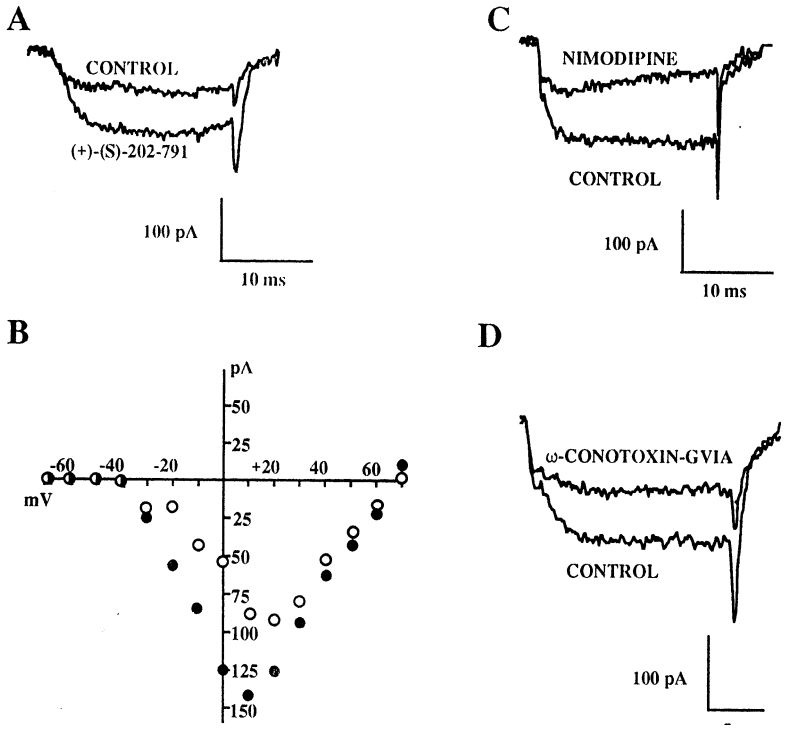

Fig. 4. L- and N-type calcium currents in CATH.a cells. (*A*) Whole-cell voltage-clamp recordings were performed on 23 CATH.a cells from 4 different platings. Superimposed membrane currents from a CATH.a cell in response to 20-msec step depolarizations to 0 mV from a holding potential of −80 mV before and after puffer application of the external solution containing 10 μM (+)-(S)-202-791. (*B*) Current-voltage relation of the cell shown in (*A*) before (open circles) and after application of 10 μM (+)-(S)-202-791 (filled circles). External solution #5; Puffer solution #3; Internal solution #3. (*C*) Whole-cell voltage-clamp recordings were performed on 10 CATH.a cells from 2 different platings. Superimposed membrane currents from a CATH.a cell in response to 20-msec step depolarizations to +10 mV from a holding potential of −80 mV before and after puffer application of the external solution containing 5 μM nimodipine. External solution #5; Puffer solution #3; Internal solution #3. (*D*) Whole-cell voltage-clamp recordings were performed on 23 CATH.a cells from 3 different platings. Superimposed membrane currents from a CATH.a cell in response to 20-msec step depolarizations to +10 mV from a holding potential of −80 mV before and after puffer application of the external solution containing 10 μM ω-conotoxin-GVIA. External solution #5; Puffer solution #3; Internal solution #3.

To test for the presence of N-type calcium currents, we employed ω-conotoxin-GVIA, a peptide from the marine snail *Conus geographus* which irreversibly blocks N-type currents (Olivera et al., 1984, 1985, 1990; Aosaki & Kasai, 1989; Plummer et al., 1989). Puffer application of 10 μM ω-conotoxin-GVIA irreversibly decreased the calcium current by 40 ± 16% in 8 out of 23 (35%) CATH.a cells (Fig. 4*D*). These results suggest that in 35% of the CATH.a cells, part of the calcium current is carried through N-type calcium channels.

The above findings suggest that specific pharmacological agents of L- and N-type channels affect distinct calcium current classes in CATH.a cells, one sensitive to dihydropyridines and the other sensitive to ω-conotoxin-GVIA. This interpretation is further supported by results of experiments in which currents were not blocked by puffer application of 10 μM ω-conotoxin-GVIA but were potentiated by subsequent application of 10 μM (+)-(S)-202-791 (dihydropyridine-sensitive/ω-conotoxin-GVIA-resistant current). Additional evidence for the existence of distinct L- and N-type channel classes comes from experiments in which calcium currents were blocked by puffer application of 10 μM ω-conotoxin-GVIA but not by the presence of 5 μM nimodipine in the external solution (ω-conotoxin-GVIA-sensitive/dihydropyridine-resistant current). The demonstration that dihydropyridine-sensitive/ω-conotoxin-GVIA-resistant and ω-conotoxin-GVIA-sensitive/dihydropyridine-resistant currents exist indicates the presence of distinct classes of L- and N-type currents, respectively.

A peptide from the funnel web spider *Agelenopsis aperta*, ω-agatoxin-IVA, blocks P-type currents in Purkinje neurons (Adams, Bindokas & Venema, 1992; Mintz et al., 1992*a*,*b*); at concentrations higher than 200 nM, ω-agatoxin-IVA may block other types of calcium current as well (Sather et al., 1993; Soong et al., 1993). Puffer application of 100–200 nM ω-agatoxin-IVA for up to 3 min did not affect the calcium current in any of 17 CATH.a cells suggesting that P-type calcium channels are not present in CATH.a cells.

HVA currents could still be detected when CATH.a cells were bathed in a solution containing 5 μM nimodipine, 10 μM ω-conotoxin-GVIA, and 200 nM ω-agatoxin-IVA, suggesting the presence of calcium channels which are not L-, or N-, or P-type. The average size of these ''resistant'' currents was 75 ± 16 pA (n = 9). Fifty-five percent of the cells (9/16) exhibited this ''resistant'' HVA current. The presence of ''resistant'' currents was further confirmed when 10 μM (+)-(S)-202-791 was puffer applied to CATH.a cells bathed in a solution containing 10 μM ω-conotoxin-GVIA and 200 nM ω-agatoxin-IVA; in 50% of the cells (4/8) exhibiting a calcium current, (+)-(S)-202-791 had no effect. The average size of these ''resistant currents'' was 85 ± 23

pH ($n = 4$), similar to the average size mentioned above from cells in a bath solution containing L-, N-, and P-type antagonists (75 ± 16 pA).

In summary, in 73% of the CATH.a cells exhibiting a calcium current, about 40% of the current was dihydropyridine-sensitive, indicating flow through L-type channels; in 35% of the cells expressing a calcium current, 40% of the current was sensitive to ω-conotoxin-GVIA, indicating the presence of N-type channels. In 55% of the cells with a HVA calcium current, approximately half of the current was resistant to a combination of L-, N-, and P-type channel antagonists, suggesting the presence of HVA channels which are not L-, N-, or P-type.

VOLTAGE-GATED CURRENTS IN CATH.A SUBCLONES

It is possible that the heterogeneity in voltage-gated currents expressed amongst different CATH.a cells was due to genetically different subpopulations within the CATH.a cultures. To test this possibility, the CATH.a cells were subcloned twice at limiting dilution in microtiter wells and those colonies that arose from a single cell were identified by visual inspection. Whole-cell recordings were then performed on four subclones. The C12C1 and A12A2 subclones were independent isolates, while A5H8 and A5G11 were sibling colonies from the same first-cycle parental clone.

Surprisingly, within each subclone, heterogeneity still persisted. However, the C12C1, A12A2, and A5H8 differed from the parental CATH.a cells. Almost all C12C1, A12A2, and A5H8 cells exhibited putative potassium currents (93%, 93%, and 100% respectively) in contrast to 66% of the parental CATH.a cells. Few (34%, 0%, and 16% respectively) expressed detectable calcium currents although 68% of the parental CATH.a cells did (Table 2*A*). Almost all C12C1, A12A2, and A5H8 cells, like the parental CATH.a cells, exhibited sodium currents (average amplitude 500–648 pA; Table 2*A* and *C*). The A5G11 line differed the most from the parental line. Only 50% of A5G11 cells exhibited a small sodium current (153 ± 127 pA; Table 2*A* and *C*). This was particularly surprising given that its sibling clone, A5H8, behaved like the parental CATH.a line and consistently exhibited a sodium current. However, the A5G11 cells were similar to the other subclones in regard to potassium (94%) and calcium (12%) current expression. The currents were observed in various combinations and amplitudes amongst individual cells within each subclone as they were in the parental CATH.a cells (Table 2*B* and *C*). In total, these results suggest that genetic heterogeneity of the parental CATH.a line can account only partially for the heterogeneity in types and levels of voltage-gated currents.

Discussion

The major finding of this study is that CATH.a cells possess voltage-gated TTX-sensitive sodium currents and HVA calcium currents similar to those found in other neuronal preparations. By extending the number of neuronal properties characterized in these cells (e.g., Suri et al., 1993), this study further supports the use of CATH.a cells for biochemical and molecular studies of CNS catecholaminergic neurons. Because CATH.a cells are noradrenergic, it is likely that they originated from the locus coeruleus or lateral tegmental nuclei. Preliminary evidence supports this contention (Duman, Terwilliger & Nestler, Soc. Neurosci. *Abstr.* 18, 343.5, 1992). CATH.a cells express corticotropin releasing factor (CRF) and vasoactive intestinal peptide (VIP) receptors which activate adenylate cyclase and express α_2 adrenergic, opiate, and neuropeptide (NPY) receptors which inhibit adenylate cyclase. These receptor responses are typical of those found in the locus coeruleus neurons *in vivo.*

We demonstrated that calcium currents in CATH.a cells are heterogeneous; they are affected by agonists and antagonists of L- and N-type currents to varying extents. In addition, a ''resistant'' HVA calcium current is detectable in some cells. This ''resistant'' current may be similar to that reported by Regan, Sah & Bean (1991) and Mintz et al. (1992*a*) in various neuronal preparations. These results suggest that CATH.a cells express L-, N-, and ''resistant''-type calcium currents.

For the purposes of this study, we characterized calcium currents as L-type based on their sensitivity to dihydropyridines and their insensitivity to N- and P-type channel blockers. Although a common characteristic of L-type channels is insensitivity to more positive holding potentials (Nowycky, Fox & Tsien, 1985*b*; Fox et al., 1987 *a,b*), the L-type channels in CATH.a cells do show voltage-dependent inactivation at a holding potential of −50 mV. However, defining calcium currents on the basis of their voltage-dependent inactivation is not absolute in all systems (Plummer et al., 1989; Cox & Dunlap, 1992).

It is possible that L-type channels in CATH.a cells are of a different molecular composition than those previously described thereby causing them to inactivate at more positive holding potentials. For example, it is possible that they contain different auxiliary subunits which affect channel function. Alternatively, the ion-conducting α subunit of L-type channels in CATH.a cells may be encoded by a novel L-type channel gene or may be an alternatively spliced product of a previously described L-type channel gene. Finally, L-type channels in CATH.a cells may differ in their post-translational modifications thereby promoting voltage dependent inactivation.

L- and N-type currents have also been defined on the

Table 2. Voltage-gated currents in CATH.a subclones

A

	CATH.a	C12C1	A12A2	A5H8	A5G11
Sodium current	100% (44/44)	87% (13/15)	100% (14/14)	100% (19/19)	50% (8/16)
Calcium current	68% (30/44)	34% (5/15)	0% (0/14)	16% (3/19)	12% (2/16)
Potassium current	66% (29/44)	93% (14/15)	93% (13/14)	100% (19/19)	94% (15/16)

B

	CATH.a	C12C1	A12A2	A5H8	A5G11
Na^+ only	9% (4/44)	0% (0/15)	7% (1/14)	0% (0/19)	0% (0/16)
Na^+,Ca^{2+}	25% (11/44)	7% (1/15)	0% (0/14)	0% (0/19)	6% (1/16)
Na^+,K^+	23% (10/44)	53% (8/15)	93% (13/14)	84% (16/19)	38% (6/16)
Na^+,Ca^{2+},K^+	43% (19/44)	27% (4/15)	0% (0/14)	16% (3/19)	6% (1/16)
K^+ only	0% (0/44)	13% (2/15)	0% (0/14)	0% (0/19)	50% (8/16)

C

Na^+ current	CATH.a	C12C1	A12A2	A5H8	A5G11
Average size of Na^+ current	633 ± 562 pA	648 ± 846 pA	605 ± 242 pA	500 ± 411 pA	153 ± 127 pA
Size range of Na^+ current	100–2500 pA	150–3500 pA	150–1200 pA	100–1750 pA	50–450 pA

Whole-cell voltage-clamp recordings were performed on subclones C12C1, A12A2, A5H8, A5G11, and the parental CATH.a cells. Cells were held at −80 mV and then given 20–30 msec-test pulses ranging from −80 to +80 mV. The numbers in parentheses indicate the actual number of cells tested. External solution #1; Internal solution #1. (*A*) Percentages of cells expressing sodium, calcium, and putative potassium currents. (*B*) Percentages of cells expressing a given combination of currents. (*C*) Average size and range of peak sodium current for the parental CATH.a cells and the four subclones.

basis of their inactivation kinetics; initially, L-type currents were defined by their slow inactivation and N-type currents by their relatively rapid inactivation (Nowycky et al., 1985*b*; Fox et al., 1987*a*,*b*). However, defining calcium currents on the basis of their inactivation kinetics is not absolute in all systems. The existence of slowly inactivating N-type currents has been demonstrated in some neurons including sympathetic neurons (Plummer et al., 1989; Jones & Marks, 1989*a*,*b*). In CATH.a cells the L-type currents are slowly inactivating. CATH.a cells express both slowly inactivating and rapidly inactivating N-type current. Upon blocking the slowly inactivating L-type current with nimodipine in Fig. 4*C*, a rapidly inactivating current is unmasked suggesting that this cell expresses an L-type current and a more rapidly inactivating N-type current. In other cells slowly inactivating N-type currents were revealed.

Different calcium channel types mediate different cellular functions such as neurotransmitter release and neuronal gene induction in various cell types. Biochemical studies using a rapid superfusion system to measure subsecond, calcium-dependent [H^3] glutamate release from rat cortical and hippocampal synaptosomes have demonstrated that release is largely blocked by ω-agatoxin-IVA, indicating that P-type channels are involved (Turner, Adams & Dunlap, 1992; Luebke, Dunlap & Turner, 1993). In hippocampal synaptosomes, ω-conotoxin-GVIA partially blocks release, suggesting that N-type channels are also involved (Luebke et al., 1993). Because the calcium-dependent glutamate release from

cortical and hippocampal synaptosomes is not completely blocked by these toxins, ''resistant''-type calcium channels may also be involved (Turner et al., 1992; Luebke et al., 1993). It is conceivable that biochemical studies using a rapid superfusion system can be performed with CATH.a cells to investigate the relative importance of L-, N-, and ''resistant''-type calcium channels to catecholamine release in these cells. CATH.a cells should be amenable to these biochemical studies. The heterogeneity of CATH.a cells should not be problematic for such studies considering that CATH.a cells are significantly less heterogeneous than rat cortical and hippocampal synaptosome preparations which have provided valuable information regarding neurotransmitter release. Furthermore, since CATH.a cells can easily be transfected (Lazaroff et al., 1995), they offer the unique opportunity to introduce genes which may affect the release pathway and alter neurotransmitter secretion.

L-type calcium channel activation during prolonged membrane depolarization has been shown to mediate induction of the c-fos gene via the cyclic-AMP response element (CRE) in PC12 cells and hippocampal neurons (*reviewed* in Sheng & Greenberg, 1990; Ghosh & Greenberg, 1995).

TH is also induced by membrane depolarization *in vivo* and in cell culture systems, however, the mechanism is less well understood (Dreyfus et al., 1986; Faucon-Biquet et al., 1989; Schalling et al., 1989; Ehrlich et al., 1990; Banerjee et al., 1992; Kilbourne et al., 1992). We have found that membrane depolarization of CATH.a cells increases TH transcription 100–200% via the CRE (*unpublished data*). Future work will involve examining the mechanism of this induction and the voltage-gated channels required.

Whether the voltage-gated sodium and calcium channel density in CATH.a cells can be increased by differentiation remains to be determined. We observed no increase in channel density with dibutyryl cAMP treatment which has been shown to increase sodium channel density in PC12 cells (Kalman et al., 1990) and promote differentiation in some neuronal cell lines (Prasad, 1975; De Laat & van der Saag, 1982; Rupniak et al., 1984). However, growth in serum-free medium resulted in a 2- to 3-fold increase in average sodium current size without affecting calcium current size, although the CATH.a cells did not appear more differentiated morphologically (*unpublished observations*). CATH.a cells lack NGF receptors and do not respond to NGF (*unpublished data*).

Among different CATH.a cells, heterogeneity exists in the types and amounts of voltage-gated currents expressed. These differences between individual cells are not primarily due to genetically distinct subpopulations since four subclones obtained from the CATH.a cells also exhibit heterogeneity in the types and amounts of voltage-gated currents expressed. It is possible that differences in channel expression are related to where cells are in the cell cycle or are due to unidentified microenvironmental cues. For example, channel expression may be induced or repressed by factors secreted by the cells or interactions between cells. It is now possible to investigate whether various growth factors influence voltage-gated channel expression in the parental CATH.a cells and the various subclones. As only 50% of the A5G11 cells express sodium currents and these currents are considerably smaller than those expressed in the other subclones, comparison of sodium channel gene regulation in the A5G11 cells to that in other subclones may provide insights regarding induction/repression of sodium current expression.

Despite some heterogeneity, the C12C1, A12A2, and A5H8 cells are more homogeneous than the parental CATH.a cells; in particular, almost all exhibit sodium and putative potassium currents whereas few have calcium currents. This greater uniformity within the subclones suggests that some heterogeneity in the parental CATH.a population may be due to genetic differences between cells. The parental CATH.a cells were originally derived from small clumps of cells (1–10 cells) because of the low efficiency of growing a colony from a single cell. The subcloning procedure employed here demanded that a single cell give rise to a colony; thus clones may have been selected that express a particular subset of channels, i.e., more potassium and less calcium current. The fact that three independent subclones were quite similar to each other argues that the subcloning procedure may have been a factor in selecting clones with a similar phenotype.

This study and previous work (Suri et al., 1993) demonstrate that CATH.a cells express a neuronal phenotype. Since the CATH.a cells can be grown continuously in large amounts, they may be useful for purifying, characterizing, and/or cloning various neuronal-specific molecules regardless of their heterogeneity. In future work, it may be possible to purify, characterize, and/or clone a ''resistant''-type calcium channel from CATH.a cells. In addition, the CATH.a cells will be used to further investigate membrane depolarization-induced expression of TH through the CRE and possibly isolate the transcription factor(s) involved. These studies are often not feasible when using small regions of dissected brain tissue or primary cultures where heterogeneity exists to a much greater extent and where the cells of interest may comprise a smaller percentage of the total population. The CATH.a cell line may contribute to our understanding of biochemical, molecular, and electrophysiological properties of CNS catecholaminergic brainstem neurons.

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References

Adams, M.E., Bindokas, V.P., Venema, V.J. 1992. Probing calcium channels with venom toxins. Neurotox '91: The Molecular Basis of Drug and Pesticide Action. I.M. Duce, editor. pp. 33–44. Elsevier Science, Amsterdam

Aosaki, T., Kasai, H. 1989. Characterization of two kinds of high-voltage-activated Ca-channel currents in chick sensory neurons. Differential sensitivity to dihydropyridines and ω-conotoxin GVIA. *Pfluegers Arch.* **414:**150–156

Banerjee, S.A., Hoppe, P., Brilliant, M., Chikaraishi, D.M. 1992. 5′ Flanking sequences of the rat tyrosine hydroxylase gene target accurate tissue-specific, developmental, and transsynaptic expression in transgenic mice. *J. Neurosci.* **12:**4460–4467

Barres, B.A., Chun, L.L.Y., Corey, D.P. 1989. Glial and neuronal forms of the voltage-dependent sodium channel: characteristics and cell-type distribution. *Neuron* **2:**1375–1388

Bean, B.P. 1984. Nitrendipine block of cardiac calcium channels: high-affinity binding to the inactivated state. *Proc. Natl. Acad. Sci. USA* **81:**6388–6392

Bean, B.P. 1989. Classes of calcium channels in vertebrate cells. *Annu. Rev. Physiol.* **51:**367–384

Byerly, L., Chase, P.B., Stimers, J.R. 1985. Permeation and interaction of divalent cations in calcium channels of snail neurons. *J. Gen. Physiol.* **85:**491–518

Catterall, W.A., Striessnig, J. 1992. Receptor sites for Ca^{2+} channel antagonists. *Trends Pharmacol. Sci.* **13:**256–262

Choi, H.K., Won, L.A., Kontur, P.J., Hammond, D.N., Fox, A.P., Wainer, B.H., Hoffmann, P.C., Heller, A. 1991. Immortalization of embryonic mesencephalic dopaminergic neurons by somatic cell fusion. *Brain Res.* **552:**67–76

Cox, D.H., Dunlap, K. 1992. Pharmacological discrimination of N-type and L-type calcium current and its selective modulation by transmitters. *J. Neurosci.* **12;**906–914

Crawford, G.D. Jr., Le, W.-D., Smith, R.G., Xie, W.-J., Stefani, E., Appel, S.H. 1992. A novel N18TG2 x mesencephalon cell hybrid expresses properties that suggest a dopaminergic cell line of substantia nigra origin. *J. Neurosci.* **12:**3392–3398

De Laat, S.W., van der Saag, P.T. 1982. The plasma membrane as a regulatory site in growth and differentiation of neuroblastoma cells. *Int. Rev. Cytol.* **74:**1–54

Dreyfus, C.F., Friedman, W.J., Markey, K.A., Black, I.B. 1986. Depolarization stimuli increase tyrosine hydroxylase in mouse locus coeruleus in culture. *Brain Res.* **379:**216–222

Dunlap, K., Luebke, J.I., Turner, T.J. 1995. Exocytotic Ca^{2+} channels in mammalian central neurons. *Trends Neurosci.* **18:**89–98

Ehrlich, M.E., Grillo, M., Joh, T.H., Margolis, F.L., Baker, H. 1990. Transneuronal regulation of neuronal gene specific gene expression in the mouse olfactory bulb. *Mol. Brain Res.* **7:**115–122

Faucon-Biquet, N., Rittenhouse, A.R., Mallet, J., Zigmond, R.E. 1989. Preganglionic nerve stimulation increases mRNA levels for tyrosine hydroxylase in the rat superior cervical ganglion. *Neurosci. Lett.* **104:**189–194

Fernandez, J.M., Fox, A.P., Krasne, S. 1984. Membrane patches and whole-cell membranes: a comparison of electrical properties in rat clonal pituitary (GH_3) cells. *J. Physiol.* **356:**565–585

Fox, A.P., Nowycky, M.C., Tsien, R.W. 1987*a*. Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. *J. Physiol.* **394:**149–172

Fox, A.P., Nowycky, M.C., Tsien, R.W. 1987*b*. Single-channel recordings of three types of calcium channels in chick sensory neurones. *J. Physiol.* **394:**173–200

Ghosh, A., Greenberg, M.E. 1995. Calcium signaling in neurons: molecular mechanisms and cellular consequences. *Science* **268:**239–247

Greene, L.A., Tischler, A.S. 1976. Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *Proc. Natl. Acad. Sci. USA* **73:**2424–2428

Hagiwara, S., Byerly, L. 1981. Calcium channel. *Annu. Rev. Neurosci.* **4:**69–125

Hagiwara, S., Byerly, L. 1983. The calcium channel. *Trends Neurosci.* **6:**189–193

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Hess, P. 1990. Calcium channels in vertebrate cells. *Annu. Rev. Neurosci.* **13:**337–356

Hess, P., Lansman, J.B., Tsien, R.W. 1984. Different modes of Ca channel gating behavior favored by dihydropyridine Ca agonists and antagonists. *Nature* **311:**538–544

Hof, R.P., Ruegg, U.T., Hof, A., Volgel, A. 1985. Stereoselectivity at the calcium channel: opposite action of the enantiomers of a 1,4-dihydropyridine. *J. Cardiovasc. Pharmacol.* **7:**689–693

Hosey, M.M., Lazdunski, M. 1988. Calcium channels: molecular pharmacology, structure and regulation. *J. Membrane Biol.* **104:**81–105

Jones, S.W., Marks, T.N. 1989*a*. Calcium currents in bullfrog sympathetic neurons. I. Activation kinetics and pharmacology. *J. Gen. Physiol.* **94:**151–167

Jones, S.W., Marks, T.N. 1989*b*. Calcium currents in bullfrog sympathetic neurons. II. Inactivation. *J. Gen. Physiol.* **94:**169–182

Kalman, D., Wong, B., Horvai, A.E., Cline, M.J., O'Lague, P.H. 1990. Nerve growth factor acts through cAMP-dependent protein kinase to increase the number of sodium channels in PC12 cells. *Neuron* **2:**355–366

Kilbourne, E.J., Nankova, B.B., Lewis, E.J., McMahon, A., Osaka, H., Sabban, D.B., Sabban, E.L. 1992. Regulated expression of the tyrosine hydroxylase gene my membrane depolarization. *J. Biol. Chem.* **267:**7563–7569

Kokubun, S., Prod'hom, B., Becker, C., Porzig, H., Reuter, H. 1986. Studies on Ca channels in intact cardiac cells: voltage-dependent effects and cooperative interactions of dihydropyridine enantiomers. *Mol. Pharmacol.* **30:**571–584

Lansman, J.B., Hess, P., Tsien, R.W. 1986. Blockade of current through single calcium channels by Cd^{2+}, Mg^{2+}, and Ca^{2+}: voltage and concentration dependence of calcium entry into the pore. *J. Gen. Physiol.* **88:**321–347

Lazaroff, M., Patankar, S., Yoon, S.-O., Chikaraishi, D.M. 1995. The cyclic AMP response element directs tyrosine hydroxylase expression in catecholaminergic central and peripheral nervous system cell lines from transgenic mice. *J. Biol. Chem.* **270:**21579–21589

Llinás, R., Sugimori, M., Hillman, D.E., Cherksey, B. 1992. Distribution and functional significance of the P-type, voltage-dependent Ca^{2+} channels in the mammalian central nervous system. *Trends Neurosci.* **15:**351–355

Luebke, J.I., Dunlap, K., Turner, T.J. 1993. Multiple calcium channel types control glutamatergic synaptic transmission in the hippocampus. *Neuron* **11:**895–902

Mandel, G., Cooperman, S.S. Maue, R.A., Goodman, R.H., Brehm, P. 1988. Selective induction of brain type II Na^+ channels by nerve growth factor. *Proc. Natl. Acad. Sci. USA* **85:**924–928

Mével-Ninio, M., Weiss, M.C. 1981. Immunofluorescence analysis of the time-course of extinction, reexpression, and activation of albu-

min production in rat hepatoma-mouse fibroblast heterokaryons and hybrids. *J. Cell. Biol.* **90:**339–350

Miller, R.J. 1992. Voltage-sensitive Ca^{2+} channels. *J. Biol. Chem.* **267:**1403–1406

Minna, J.D., Yavelow, J., Coon, H.G. 1975. Expression of phenotypes in hybrid somatic cells derived from the nervous system. *Genetics* **79:**373–383

Mintz, I.M., Adams, M.E., Bean, B.P. 1992*a*. P-type calcium channels in rat central and peripheral neurons. *Neuron* **9:**85–95

Mintz, I.M., Venema, V.J., Swiderek, K.M., Lee, T.D., Bean, B.P., Adams, M.E. 1992*b*. P-type calcium channels blocked by the spider toxin ω-Aga-IVA. *Nature* **355:**827–829

Nachshen, D.A. 1984. Selectivity of the Ca binding site in synaptosome Ca channels: inhibition of Ca influx by multivalent metal cations. *J. Gen. Physiol.* **83:**941–967

Nowycky, M.C., Fox, A.P., Tsien, R.W. 1985*a*. Long-opening mode of gating of neuronal calcium channels and its promotion by the dihydropyridine calcium agonist Bay K 8644. *Proc. Natl. Acad. Sci. USA* **82:**2178–2182

Nowycky, M.C., Fox, A.P., Tsien, R.W. 1985*b*. Three types of neuronal calcium channel with different calcium agonist sensitivity. *Nature* **316:**440–443

Olivera, B.M., Gray, W.R., Zeikus, R., McIntosh, J.M., Varga, J., Rivier, J., de Santos, V., Cruz, L.J. 1985. Peptide neurotoxins from fish-hunting cone snails. *Science* **230:**1338–1343

Olivera, B.M., McIntosh, J.M., Cruz, L.J., Luque, F.A., Gray, W.R. 1984. Purification and sequence of a presynaptic peptide toxin from *Conus geographus* venom. *Biochemistry* **23:**5087–5090

Olivera, B.M., Rivier, J., Clark, C., Ramilo, C.A., Corpuz, G.P., Abogadie, F.C., Mena, E., Woodward, S.R., Hillyard, D.R., Cruz, L.J. 1990. Diversity of *Conus* neuropeptides. *Science* **249:**257–263

Plummer, M.R., Logothetis, D.E., Hess, P. 1989. Elementary properties and pharmacological sensitivities of calcium channels in mammalian peripheral neurons. *Neuron* **2:**1453–1463

Prasad, K.N. 1975. Differentiation of neuroblastoma cells in culture. *Biol. Rev.* **50:**129–165

Regan, L.J., Sah, D.W.J., Bean, B.P. 1991. Ca^{2+} channels in rat central and peripheral neurons: high-threshold current resistant to dihydropyridine blockers and ω-conotoxin. *Neuron* **6:**269–280

Rupniak, H.T., Rein, G., Powell, J.F., Ryder, T.A., Carson, S., Povey, S., Hill, B.T. 1984. Characteristics of a new human neuroblastoma cell line which differentiates in response to cyclic adenosine 3′:5′-monophosphate. *Cancer Res.* **44:**2600–2607

Sanguinetti, M.C., Kass, R.S. 1984. Voltage-dependent block of calcium current in the calf cardiac Purkinje fiber by dihydropyridine calcium channel antagonists. *Cir. Res.* **55:**336–348

Sather, W.A., Tanabe, T., Zhang, J.-F., Mori, Y., Adams, M.E., Tsien, R.W. 1993. Distinctive biophysical and pharmacological properties of class A (BI) calcium channel α_1 subunits. *Neuron* **11:**291–303

Schalling, M., Stieg, P.E., Linguist, C., Goldstein, M., Hökfelt, T. 1989. Rapid increase in enzyme and peptide mRNA in sympathetic ganglia after electrical stimulation in humans. *Proc. Natl. Acad. Sci. USA* **86:**4302–4305

Sheng, M., Greenberg, M.E. 1990. The regulation and function of c-fos and other immediate early genes in the nervous system. *Neuron* **4:**477–485

Snutch, T.P., Reiner, P.B. 1992. Ca^{2+} channels: diversity of form and function. *Curr. Opin. Neurobiol.* **2:**247–253

Soong, T.W., Stea, A., Hodson, C.D., Dubel, S.J., Vincent, S.R., Snutch, T.P. 1993. Structure and functional expression of a member of the low voltage-activated calcium channel family. *Science* **260:**1133–1136

Stuhmer, W., Methfessel, C., Sakmann, B., Noda, M., Numa, S. 1987. Patch clamp characterization of sodium channels expressed from rat brain cDNA. *Eur. Biophys. J.* **14:**131–138

Suri, C., Fung, B.P., Tischler, A.S., Chikaraishi, D.M. 1993. Catecholaminergic cell lines from the brain and adrenal glands of tyrosine hydroxylase-SV40 T antigen transgenic mice. *J. Neurosci.* **13:**1280–1291

Tsien, R.W., Ellinor, P.T., Horne, W.A. 1991. Molecular diversity of voltage-dependent Ca^{2+} channels. *Trends Pharmacol. Sci.* **12:**349–354

Tsien, R.W., Lipscombe, D., Madison, D.V., Bley, K.R., Fox, A.P. 1988. Multiple types of neuronal calcium channels and their selective modulation. *Trends Neurosci.* **11:**431–438

Turner, T.J., Adams, M.E., Dunlap, K. 1992. Calcium channels coupled to glutamate release identified by ω-Aga-IVA. *Science* **258:**310–313