Multiple Types of Calcium Channels in Acutely Isolated Rat Neostriatal Neurons

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Voltage-activated high- and low-threshold Ca^{2+} currents were studied using whole-cell voltage-clamp techniques and fura-2 fluorescence measurements of intracellular Ca^{2+} in neurons acutely isolated from rat neostriatum. High-threshold Ca^{2+} currents activated around -40 mV and were present in at least 95% of neostriatal neurons. The maximum current, 736 ± 44 pA (mean $\pm$ SEM, $n = 141$), was observed around 0 mV. In 70% of neurons, high-threshold Ca^{2+} currents exhibited both inactivating and noninactivating components. The majority of the high-threshold Ca^{2+} currents appeared to belong neither to the “L-type” nor the “N-type” classification, since ω -conotoxin ($5 \mu\text{M}$) decreased this current by only 29% and nimodipine ($10 \mu\text{M}$) decreased the noninactivating component of this current by only 17%.

A low-threshold transient (T-type) Ca^{2+} current was observed in 40% of neurons. When both T-type and high-threshold Ca^{2+} currents were present, their maximum amplitudes were 78 ± 7 pA and 800 ± 57 pA, respectively (mean $\pm$ SEM, $n = 58$). At a holding potential of -100 mV, the T-type Ca^{2+} current activated around -60 mV, with maximum current near -40 mV. Steady-state inactivation of the T-type Ca^{2+} current was observed at holding potentials positive to -125 mV, and the current was half-inactivated at -88 mV. Recovery from inactivation to 90% of maximum occurred within 800 msec. Mn^{2+} or Co^{2+} (3 mM) blocked both high-threshold and T-type Ca^{2+} currents, whereas Cd^{2+} ($25 \mu\text{M}$) or verapamil ($50 \mu\text{M}$ and $150 \mu\text{M}$) preferentially blocked high-threshold over T-type Ca^{2+} currents.

In response to depolarization by 50 mM K^+ , fura-2 fluorescence measurements showed increased intracellular Ca^{2+} in both the soma and the proximal dendrites of neostriatal neurons that was markedly reduced by $25 \mu\text{M Cd}^{2+}$. These findings suggest that high-threshold Ca channels are present in both the soma and proximal dendrites of neostriatal neurons.

[Key words: striatum, calcium, fura-2, ion channels, nimodipine, verapamil, conotoxin, acute isolation, voltage-clamp, imaging, basal ganglia]

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Neuronal voltage-dependent Ca channels are involved in the mediation of intracellular processes such as neurotransmitter release, enzyme activation, gene expression, and excitotoxicity. In addition, the kinetic properties and spatial distribution of Ca channels on the cell surface are major determinants of the firing patterns and input–output characteristics of various neuronal cell types (Llinás, 1988). There is substantial electrophysiological and pharmacological evidence for multiple types of voltage-dependent Ca channels (Carbone and Lux, 1984; Nowycky et al., 1985; Fox et al., 1987; Bean, 1989). Low-voltage-activated Ca channels have been referred to as LVA or “T-type” (transient), depending on the classification scheme (Carbone and Lux, 1984; Fox et al., 1987; Tsien et al., 1988; Swandulla et al., 1991). High-voltage-activated Ca channels (HVA; Carbone and Lux, 1984) have been subdivided by various authors into “L” (long-lasting), “N” (neither T nor L), and “P” (Purkinje) subtypes according to kinetic and pharmacological criteria (Fox et al., 1987; Tsien et al., 1988; Llinás et al., 1989). However, recent studies indicate that the biophysical and pharmacological classification of different types of HVA Ca channels is not as straightforward as was initially thought (Aosaki and Kasai, 1989; Plummer et al., 1989; reviewed by Hess, 1990; Sher and Clementi, 1991; Bertolino and Llinás, 1992). Indeed, molecular biological approaches suggest that at least eight different Ca channels are expressed in the brain (Snutch et al., 1990), and there is evidence that these Ca channels are differentially distributed among various CNS regions (Regan et al., 1991).

The neostriatum is the primary site of afferent input to the basal ganglia. The firing patterns of neostriatal output neurons have an important influence on the functioning of the extrapyramidal motor system (Gerfen, 1992). The presence of voltage-dependent Ca channels in rat neostriatal neurons has been inferred from current-clamp studies (Kita et al., 1985b; Misgeld et al., 1986; Calabresi et al., 1987, 1990) and single-electrode voltage-clamp studies (Cherubini and Lanfume, 1987). There is general agreement that neostriatal neurons possess high-threshold Ca channels capable of generating Ca^{2+} spikes. It has been shown that these Ca^{2+} spikes are prolonged by the “L-type” Ca channel agonist Bay K 8644 (Cherubini and Lanfume, 1987). Physiological evidence for the existence of low-threshold Ca channels (Calabresi et al., 1987; Cherubini and Lanfume, 1987) is less compelling. Neostriatal neurons do not exhibit rhythmic burst discharge patterns (Calabresi et al., 1987; Galarraga et al., 1989) characteristic of neurons with prominent low-threshold Ca^{2+} spikes (Llinás and Yarom, 1981a; Jahnsen and Llinás, 1984; Llinás, 1988). Although both low- and high-threshold Ca channels have recently been demonstrated in cultured fetal neostriatal neurons (Bargas et al., 1991), the size and types of Ca^{2+} currents exhibited by neurons in culture can be

influenced profoundly by cell culture conditions (Yaari et al., 1987; Doerner et al., 1988; Plummer et al., 1989; Boland and Dingledine, 1990).

In the present study, Ca^{2+} currents recorded from acutely isolated adult rat neostriatal neurons were analyzed using whole-cell voltage clamp. The use of cells acutely isolated from the intact environment of the nervous system avoids the above problems associated with cultured neurons. Moreover, the limited dendritic arborization of acutely isolated neurons significantly improves uniformity of voltage control throughout the neuron. Three main questions are addressed in this article. (1) What types of Ca^{2+} currents are present in neostriatal neurons, and what are their distribution and relative amplitude? (2) What are the pharmacological characteristics of Ca^{2+} currents in the neostriatum? (3) Is there a preferential localization of high-threshold Ca^{2+} currents in the soma or proximal dendrites of neostriatal neurons? Our results indicate that the majority of high-threshold Ca^{2+} currents in neostriatal neurons appear to be neither "N-" nor "L-type." We also present evidence that high-threshold Ca channels are present in both the soma and proximal dendrites of neostriatal neurons.

Some of these results have been presented in preliminary form (Hoehn et al., 1991).

Materials and Methods

Cell preparation. Neostriatal neurons were isolated using a technique adapted from Kay and Wong (1986). Male Sprague-Dawley rats (21–28 d postnatal) were decapitated and their brains removed rapidly and immersed in ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM) 124 NaCl, 5 KCl, 26 NaHCO_3 , 2 CaCl_2 , 1.3 MgCl_2 , and 10 D-glucose (pH 7.4, ~280 mOsm) equilibrated with 95% O_2 , 5% CO_2 . The cerebrum was bisected in the coronal plane with a razor blade and the anterior half glued and secured with blocks of 4% low-melt agarose to a wedge of Plexiglas inclined at 30°. Transverse slices (400 μm thick, 30° rostral-up from the horizontal) containing neostriatum as well as overlying frontal cortex were cut with a vibratome. Slices were transferred to a spinner flask containing 30 ml of low- Ca^{2+} (0.1 mM), high- Mg^{2+} (3.2 mM) aCSF at 33°C to which 1 mM kynurenic acid, 40 mg of trypsin (9000 U/mg), and 20 mg of hyaluronidase (1500 U/mg) were added. The slices were stirred gently with a magnetic stirrer at a rate sufficient to prevent them from settling and the solution was bubbled continuously with 95% O_2 , 5% CO_2 . After 90 min, slices were rinsed with 60 ml of aCSF and transferred for storage to a holding chamber filled with continuously bubbled aCSF at 21°C. Slices were used within 2–8 hr of preparation. When needed, a slice was transferred to HEPES-buffered Dulbecco's Modified Eagle's Medium (DMEM) containing 1 mM kynurenic acid and 0.65 mg/ml of trypsin inhibitor (50 U/mg). Under a dissecting microscope, the neostriatum was isolated with a scalpel blade and cut into 1–2 mm² blocks, and the cells were dissociated by trituration in 0.5 ml DMEM solution using a fire-polished Pasteur pipette with an approximate tip diameter of 1 mm. For fura-2 imaging experiments, the DMEM also contained the acetoxymethyl form of fura-2 (fura-2/AM; Molecular Probes) to load the cells with the Ca^{2+} -indicator dye. The suspension of dissociated cells was then transferred to a 0.5 ml perfusion chamber mounted on a Zeiss IM-35 inverted microscope. Neurons were allowed to settle on poly-L-lysine-coated coverslips.

Electrophysiological recording. Ten minutes after cells were placed on the coverslips, the DMEM was replaced via superfusion at 1–1.5 ml/min with the extracellular recording solution containing (in mM) 90 NaCl, 40 tetraethylammonium chloride (TEA-Cl), 10 HEPES, 5 CaCl_2 , 4 4-aminopyridine, and 0.0009 tetrodotoxin (TTX). The solution was continuously bubbled with 100% O_2 and maintained at room temperature (pH 7.4, ~275 mOsm). Some studies were conducted in 0 mM NaCl, 130 mM TEA-Cl-containing extracellular solution that was otherwise identical to the one described above. All data illustrated in the figures were obtained in Na^+ -free extracellular medium except those in Figure 6.

The whole-cell variation of the patch-clamp technique was used (Hamill

et al., 1981). Patch electrodes were pulled from thin-walled glass (1.5 mm o.d.; TW 150 F-4, World Precision Instruments) in four stages on a Flaming-Brown micropipette puller (model P-87). The intracellular solution contained (in mM) 70 trizma phosphate, 25 trizma base, 40 TEA-Cl, 11 EGTA, 2.0 Mg-ATP, and 0.3 Na-GTP (pH 7.30, ~270 mOsm). To decrease the rundown of Ca^{2+} currents (Forscher and Oxford, 1985), 20 mM phosphocreatine and 50 U/ml of creatine phosphokinase were added to the intracellular solution in some experiments and the concentration of trizma phosphate was decreased to 52 mM to maintain osmolality. Creatine phosphokinase, Mg-ATP, Na-GTP, and phosphocreatine were added to the intracellular solution just prior to use. No difference in Ca^{2+} current characteristics was noted with the two solutions except for a small decrease in the rate of high-threshold Ca^{2+} current rundown. Electrodes filled with this solution had a resistance of 3–7 M Ω when tested in recording solution. A 7 mV junction potential was measured between the electrode and bath solution. Voltage command levels were not compensated and are thus shifted 7 mV in the positive direction.

Current recordings were obtained using a single-electrode voltage-clamp amplifier (Axopatch-1D) and were filtered at 5 kHz (–3 dB point). Step depolarizations from the holding potential were applied at intervals of 3–10 sec to allow Ca channels to recover from inactivation. Compensation circuitry was used to minimize series resistance errors (R_s) and 50–80% of the series resistance could be compensated. Data were digitized and analyzed using pCLAMP software (Axon Instruments). All currents were leak subtracted by one of two methods. In most cases, a linear leak component was digitally subtracted from current records in order to isolate the Ca^{2+} current. In some cases (as indicated in the figures), an inactivating component of the Ca^{2+} current was isolated and leak and capacitive currents were eliminated by subtracting currents obtained from a holding potential of –60 mV (where inactivation of T-type Ca^{2+} currents was largely complete) from currents obtained from a holding potential of –100 mV (where inactivation was minimal). In some neurons, Ca^{2+} currents could not be clamped, as judged by the lack of smoothly voltage-dependent current activation and a delay in onset of the currents. These neurons generally had longer processes and somewhat higher whole-cell capacitances than the cells in which Ca^{2+} currents could be clamped and were not included in the analysis.

Intracellular Ca^{2+} imaging. The acetoxymethyl form of fura-2 (50 μg) was dissolved in dimethyl sulfoxide with pluronic acid, added to 3 ml of DMEM containing 1 mM kynurenic acid and 0.6 mg/ml trypsin inhibitor, and then sonicated to ensure that the dye was dispersed. Cells isolated in fura-containing DMEM were allowed to settle on coverslips for 20 min while in fura-containing DMEM. The DMEM was then replaced by superfusing the bath volume (500 μl) with continuously bubbled aCSF at 1.0–1.5 ml/min. Experiments were performed at room temperature. When extracellular K^+ was increased, NaCl was replaced with an equal amount of KCl.

Cells were observed with a 63 $\times$ plan-neofluar water immersion objective (Zeiss; 1.25 NA) and were illuminated using a 75 W xenon lamp powered by a stabilized power supply. For fura-2 imaging, cells were illuminated at 340 nm or 380 nm and video images were obtained by a CCD camera (MTI CCD-72) coupled to an image intensifier (KS-1381, Video Scope). Video images (averages of eight frames obtained at 30 Hz) were digitized to 512 $\times$ 480 pixel arrays and were analyzed with an Imaging Technology 151 image processor (Cambridge, MA) controlled by a computer (Packard-Bell 386). Pixel ratios of 340:380 nm fluorescence intensities were calculated.

Materials. All inorganic salts were purchased from Fisher Scientific (Fair Lawn, NJ). Trypsin (type XI), hyaluronidase (type V), Mg-ATP, Na-GTP (type IIS), di(Tris)-phosphocreatine, creatine phosphokinase (from bovine heart, type III), $\pm$ -verapamil hydrochloride, kynurenic acid, TTX, TEA-Cl, 4-aminopyridine, ω -conotoxin GVIA (70% peptide content), and dimethyl sulfoxide were purchased from Sigma Chemical Co. (St. Louis, MO); trypsin inhibitor (from chicken egg white) was purchased from Boehringer Mannheim (Laval, Quebec, Canada); and fura-2/AM and pluronic-F-127 were purchased from Molecular Probes (Eugene, OR). DMEM was purchased from GIBCO Laboratories (Grand Island, NY). Ethosuximide was a gift from Parke-Davis (Brockville, Ontario, Canada), and nimodipine was a gift from Dr. Alexander Scriabine, Miles Institute for Preclinical Research (West Haven, CT).

Drugs were added hyperosmotically to the extracellular medium from 1000-fold concentrated stock solutions in distilled H_2O . Exceptions to this were nimodipine, which was dissolved in absolute ethanol, and ω -conotoxin, which was dissolved in distilled H_2O containing 1 mg/ml

Figure 1. Photomicrographs of acutely isolated striatal neurons. In *A* and *C*, the recording electrode can be seen following formation of a tight seal on the cell membrane. Scale bar applies to all panels.

bovine serum albumin. Equivalent concentrations of ethanol or bovine serum albumin alone did not affect Ca^{2+} currents. Aliquots of stock solutions of verapamil, nimodipine, and ω -conotoxin were kept frozen, and nimodipine was kept in the dark until immediately prior to use.

Results

Acute isolation of neostriatal neurons

Examples of the morphology of neostriatal neurons following the dissociation procedure are shown in Figure 1. As reported in histological studies (Chang et al., 1982), neostriatal neurons exhibited a continuous range of cell soma size. The majority (greater than 95%) of the cell somata of dissociated neostriatal neurons measured 10–20 μm across their widest diameter, thus corresponding in size and abundance to medium spiny striatal neurons (Mensah and Deadwyler, 1974; Chang et al., 1982). In addition, dissociation yielded some larger neurons with soma

diameters up to 40 μm . Obvious differences in current properties between neurons of different sizes were not observed. For consistency, the majority of neurons included in the present study corresponded in size to medium spiny neurons and had only one or two thin primary dendrites.

Ca^{2+} current characteristics and distribution among neostriatal neurons

The characteristics and distribution of Ca^{2+} currents were studied in 210 acutely isolated neostriatal neurons using intra- and extracellular solutions designed to block Na^{+} and K^{+} currents and reveal Ca^{2+} currents (see Materials and Methods). Three additional lines of evidence show that the currents studied were indeed Ca^{2+} currents: (1) in 4 of 4 neurons the Ca^{2+} current was decreased substantially when 5 mM CaCl_2 was substituted by 1

Figure 2. Diversity of high-threshold Ca^{2+} currents in striatal neurons. *A* and *B* illustrate data from a cell representative of 30% of striatal neurons in that it exhibited only the HVA_s type of high-threshold Ca^{2+} current. Ca^{2+} current recordings in *A* illustrate the absence of steady-state inactivation. Currents elicited by 150 msec steps to the test potential (-10 mV) were not affected by a change in holding potential from -100 mV ($\circ$) to -60 mV ($\blacktriangle$). Similarly, the peak Ca^{2+} current-voltage relationship illustrated in *B* was not affected by the change in holding potential. *C* and *D* illustrate data from a cell representative of 70% of striatal neurons in that it had both high-threshold sustained (HVA_s) Ca^{2+} currents and high-threshold inactivating (HVA_i) Ca^{2+} currents. Current recordings in *C* show the different amplitude and shape of Ca^{2+} currents elicited from holding potentials of -100 mV and -60 mV . All currents in this and subsequent figures (except Fig. 6) were leak subtracted. The inactivating current component of the high-threshold Ca^{2+} current (HVA_i , $C1-C2$) was isolated by subtracting the current obtained from a holding potential of -60 mV from the current obtained from a holding potential of -100 mV . The peak Ca^{2+} current-voltage relationship is illustrated in *D*. Note that the test potential at which the maximum Ca^{2+} current was observed (-10 mV) did not change. The broken lines indicate 0 current.

mm CaCl_2 , 10 mm MgCl_2 -containing solution; (2) in 34 of 34 additional neurons studied, Ba^{2+} (2.5 or 5 mm) supported current through these channels; and (3) the currents were blocked by inorganic and organic Ca channel blockers as reported below.

Different Ca^{2+} currents were distinguished primarily on the basis of differences in activation range [high threshold (HVA) beginning around -40 mV and low threshold around -60 mV] and the presence or absence of steady-state inactivation as determined by a change in amplitude of the Ca^{2+} current following a change in holding potential from -100 mV to -60 mV . As in other types of neurons (Nowycky et al., 1985; Tsien et al., 1988), three types of Ca^{2+} currents were observed in acutely isolated neostriatal neurons. A *sustained* HVA Ca^{2+} current (HVA_s) was defined as the current that activated around -40 mV and persisted when elicited from a holding potential of -60 mV . An *inactivating* HVA Ca^{2+} current (HVA_i) was defined as the current that activated around -40 mV but was inactivated by a change in holding potential from -100 mV to -60 mV . The third type of Ca^{2+} current, the low-threshold transient (T-type) current, activated around -60 mV and was also inactivated by a change in holding potential from -100 mV to -60 mV . As defined above, the kinetics of the HVA_s , HVA_i , and T-type currents correspond roughly to the "L," "N," and "T" Ca^{2+} currents defined by Tsien and colleagues (Nowycky et al.,

1985; Tsien et al., 1988). However, the results of pharmacological experiments in neostriatal neurons argue against a direct correspondence between the HVA_s and HVA_i currents and "L" and "N" currents (see below).

High-threshold Ca^{2+} currents were present in over 95% of neurons (Fig. 2). These currents typically activated around -40 mV and reached a maximum amplitude of $736 \pm 41\text{ pA}$ (mean $\pm$ SEM, 141 neurons) between -10 mV and 0 mV . Figure 2, *A* and *B*, illustrates Ca^{2+} currents recorded from a neuron typical of 30% of neostriatal neurons in that it exhibited only the HVA_s type of high-threshold Ca^{2+} current. The remaining 70% of neurons (35 of 51) exhibited both the HVA_s and HVA_i components of high-threshold Ca^{2+} currents as indicated by a decrease in amplitude of the high-threshold Ca^{2+} current when it was elicited from less negative holding potentials (Fig. 2*C,D*). The voltages at which the maximum current amplitude and the activation of the high-threshold currents occurred did not change with a change in holding potential from -100 mV to -60 mV (Fig. 2*B,D*). In the 70% of neurons exhibiting an HVA_i component, the amplitude of the maximum current (measured at either -10 mV or 0 mV) decreased from $881 \pm 101\text{ pA}$ to $683 \pm 95\text{ pA}$ (mean $\pm$ SEM, $n = 35$) with a change in holding potential from -100 mV to -60 mV . Therefore, when present, the HVA_i Ca^{2+} current represented only about 23% of the total

Figure 3. An example of the T-type Ca^{2+} currents that were present in 40% of striatal neurons. **A**, Current recordings of 150 msec steps to test potentials (V_{test}) shown. The left column (○) illustrates combined HVA_s and T-type Ca^{2+} currents obtained from a holding potential of -100 mV. Note the presence of both an inactivating component of Ca^{2+} current and a sustained Ca^{2+} current that reaches maximum at 0 mV. The right column (●) illustrates inactivating current that was isolated by subtracting currents obtained from a holding potential of -60 mV from currents obtained from a holding potential of -100 mV. In this cell the inactivating current appears to consist only of the T-type Ca^{2+} current and there is no evidence for an HVA_s Ca^{2+} current. **B**, The current-voltage relationship of the peak Ca^{2+} currents from **A** illustrates the two separate maxima corresponding to the T-type and HVA_s Ca^{2+} currents. The broken lines indicate 0 current.

high-threshold Ca^{2+} current in neostriatal neurons. HVA_s Ca^{2+} currents exhibited minimal inactivation during the 150 msec depolarization. Inactivation of HVA_s currents during the 150 msec depolarization was variable and ranged from minimal (e.g., Fig. 2C) to almost complete inactivation (see Fig. 4A).

In 40% (58 of 146) of neostriatal neurons a T-type Ca^{2+} current was observed from a holding potential of -100 mV (Fig. 3). This current began to activate around -60 mV, and the peak current amplitude at -40 mV or -30 mV was 78 ± 8 pA (mean

$\pm$ SEM, $n = 58$). The T-type Ca^{2+} current was totally inactivated at a holding potential of -60 mV.

Some neurons possessed all three types of Ca^{2+} currents (Fig. 4). A possible correlation between the size of neostriatal neurons and the types of Ca^{2+} currents present was tested. Neurons were grouped according to capacitance, into small (≤ 6 pF, 39 of 146 neurons), medium (7–13 pF, 68 of 146 neurons), and large (≥ 14 pF, 39 of 146 neurons) neurons. The density of the combined high-threshold Ca^{2+} currents elicited from a holding potential

of -100 mV was 89 ± 11 pA/pF ($n = 39$), 68 ± 5 pA/pF ($n = 68$), and 65 ± 5 pA/pF ($n = 39$) in small, medium, and large neurons, respectively. Therefore, the distribution and magnitude of high-threshold Ca^{2+} currents among neurons of various sizes were not obviously different. There was, however, a difference in distribution of T-type Ca^{2+} currents in that a larger percentage of small neurons possessed these currents (45%, 40%, and 33% in small, medium, and large neurons, respectively), and when present they represented a greater fraction of the total cell Ca^{2+} current. The maximum magnitude of the T-type Ca^{2+} currents remained relatively constant at 89.7 ± 14.6 pA, 61.5 ± 8.3 pA, and 94.2 ± 11.7 pA in small, medium, and large neurons, respectively. However, when compared in size to the maximum total high-threshold Ca^{2+} current elicited from a holding potential of -100 mV, the T-type Ca^{2+} current was 21% of the size of high-threshold Ca^{2+} currents in small neurons, 9% of the size of high-threshold Ca^{2+} currents in medium-sized neurons, and only 8% of the size of high-threshold Ca^{2+} currents in large neurons. Thus, the density of the T-type Ca^{2+} was 17 ± 3 pA/pF ($n = 18$), 7 ± 1 pA/pF ($n = 27$), and 6 ± 1 pA/pF ($n = 13$) in small-, medium-, and large-sized neurons, respectively.

Kinetics of activation and inactivation of Ca^{2+} currents

Steady-state inactivation of the T-type Ca^{2+} current was measured by varying the holding potential from -125 mV to -50 mV for 3 sec prior to activating the T-type Ca^{2+} current with a test potential of -40 mV (Fig. 5). At this potential the T-type Ca^{2+} current could be observed in virtual isolation from the high-threshold Ca^{2+} currents. Steady-state inactivation was observed when the holding potential was positive to -125 mV. The current was half-inactivated at -88 mV and was totally inactivated at -60 mV. The fractional current, normalized to the maximum current, was smoothly voltage dependent and was fit with the Boltzmann equation

$$I/I_{\max} = \{1 + \exp[-(V - V_{0.5})/k]\}^{-1},$$

where I is peak current at V , $I_{\max}$ is the maximum current from a holding potential of -125 mV, V is the holding potential, $V_{0.5}$ is the half-inactivation value (-87.6 mV), and k is the slope constant, which in this case was found to be -6.1 mV.

The voltage dependence of activation of the T-type Ca^{2+} current was examined by varying test potential pulses from a constant holding potential of -100 mV. The T-type Ca^{2+} current was isolated by subtraction as described earlier, in neurons that did not exhibit an HVA, Ca^{2+} current. The curve was smoothly voltage dependent. The best fit of the curve with the Boltzmann equation was obtained when $V_{0.5} = -52.8$ mV and $k = 5.8$ mV. In a similar manner, the voltage dependence of activation of the HVA, Ca^{2+} current was obtained by varying test potentials from a holding potential of -60 mV. The activation curve of the HVA, Ca^{2+} current was also smoothly voltage dependent and was best fit by the Boltzmann equation when $V_{0.5} = -21.6$ mV and $k = 5.6$ mV.

The recovery from inactivation of the T-type Ca^{2+} current was also examined and the results are shown in Figure 6. These studies were conducted by selecting neurons with a prominent T-type Ca^{2+} current and repolarizing them to -100 mV from a holding potential of -60 mV for various time periods before eliciting the low-threshold Ca^{2+} current in relative isolation with a test pulse to -40 mV. Recovery to 90% was seen after 800 msec of repolarization.

Figure 4. An example of a striatal neuron exhibiting all three types of Ca^{2+} currents. *A*, Current recordings of high-threshold Ca^{2+} currents obtained by a 150 msec step to the test potential (-10 mV) from holding potentials of -100 mV ($\circ$) and -60 mV ($\blacktriangle$). The inactivating current ($\bullet$, A1-A2) was obtained by subtraction as described in the Figure 3 caption. The broken lines indicate 0 current. In this cell the HVA, current exhibits inactivation during the 150 msec step. *B*, Current-voltage relation of peak Ca^{2+} currents for the same cell illustrated in *A*. Note the two separate inflections of the inactivating current corresponding to the T-type (-50 mV) and HVA, (-10 mV) Ca^{2+} currents.

Pharmacological characteristics of Ca^{2+} currents in neostriatal neurons

The effects of the divalent cations Mn^{2+} , Co^{2+} , and Cd^{2+} , which are known Ca channel blockers, were determined on neostriatal Ca^{2+} currents. In three neurons that had both high-threshold and T-type Ca^{2+} currents, both currents were blocked to a similar degree by Mn^{2+} (Fig. 7). Thus, 1 mM Mn^{2+} decreased Ca^{2+} currents by about 50% (high-threshold currents decreased $54 \pm 6\%$, $n = 4$) and 3 mM Mn^{2+} blocked almost all of these currents (high-threshold Ca^{2+} currents decreased by $93 \pm 3\%$, $n = 7$). This effect was largely reversible and was accompanied by a shift of about 10 mV in the positive direction of the voltage eliciting the peak current. Co^{2+} also decreased both high- and low-threshold Ca^{2+} currents at 1 mM and 2 mM concentrations ($n = 3$; data not shown), with the block virtually complete at 3 mM ($n = 3$, data not shown). The higher concentration (3 mM) of Co^{2+} also appeared detrimental to cell viability as judged by

Figure 5. Voltage dependence of inactivation of the T-type Ca^{2+} current and of activation of the T-type and HVA Ca^{2+} currents. *A*, Current recordings from a representative experiment to determine steady-state inactivation of the T-type Ca^{2+} current using the voltage protocol illustrated below the current traces. The holding potential was held for 3 sec at values that varied between -125 mV and -50 mV prior to evoking the T-type Ca^{2+} current with a test potential of -40 mV. *B*, Plot of inactivation of the T-type Ca^{2+} current (∇ , $n = 4$ neurons), activation of the T-type Ca^{2+} current ($\circ$, $n = 7$ neurons) and activation of the HVA Ca^{2+} current ($\bullet$, $n = 7$ neurons). Values are mean $\pm$ SEM of normalized data with fractional current plotted as $I/I_{\max}$. The voltage dependence of activation of the T-type Ca^{2+} current was determined from a holding potential of -100 mV with steps to test potentials between -90 mV and 0 mV and the inactivating current was isolated as described in the Figure 3 caption. The voltage dependence of activation of the HVA Ca^{2+} current was obtained from a holding potential of -60 mV with steps to test potentials between -50 mV and $+30$ mV. The data points were fit by Boltzmann relations (see text). The voltage at which half-maximal inactivation of the T-type Ca^{2+} current occurred was -87.6 mV, and the slope of the equation (k) was -6.1 mV. The voltages at which half-maximal activation of the T-type and HVA Ca^{2+} currents occurred were -52.8 mV and -21.6 mV, and the slopes (k) were 5.8 and 5.6 mV, respectively.

Figure 6. Time course of recovery from inactivation of the T-type Ca^{2+} current. *A*, Current recordings from a representative experiment using the voltage protocol illustrated below the current recordings. The holding potential was stepped from -60 mV to -100 mV for variable durations between 50 and 1200 msec (Δt) before T-type Ca^{2+} currents were evoked by a step to -40 mV. Currents were not leak subtracted. The amplitude of the T-type Ca^{2+} current was determined by subtracting the maximum current from the steady-state current at the end of the step to -40 mV. *B*, The fractional recovery of the T-type Ca^{2+} current is plotted with respect to time. Symbols represent individual data points from six different neurons, with each type of symbol representing a different experiment. The solid line is a single exponential with $\gamma = 288$ msec.

an increase in the holding current. In contrast to the nonspecific block by Mn^{2+} and Co^{2+} of high-threshold and T-type Ca^{2+} currents, a low concentration of Cd^{2+} ($25 \mu\text{M}$) reversibly blocked high-threshold Ca^{2+} currents by $79 \pm 3\%$ ($n = 14$) while leaving T-type Ca^{2+} channels largely unaffected (decreased $14 \pm 5\%$, $n = 7$). A representative experiment illustrating the differential effect of $25 \mu\text{M}$ Cd^{2+} on high-threshold Ca^{2+} current and on T-type Ca^{2+} current is shown in Figure 8.

The organic Ca channel blocker verapamil also preferentially blocked high-threshold Ca^{2+} currents as compared to T-type Ca^{2+} currents (Fig. 9) and this effect was partially reversible. At

Figure 7. Effect of Mn^{2+} on high-threshold and T-type Ca^{2+} currents. *A* and *B*, Representative current recordings obtained by stepping from a holding potential of -100 mV to the indicated test potentials to illustrate the effect of Mn^{2+} on high-threshold (*A*) and T-type (*B*) Ca^{2+} currents. The cell was sequentially exposed to control medium ($\circ$), 1 mM Mn^{2+} ($\blacklozenge$), 3 mM Mn^{2+} ($\blacktriangle$), and control medium (washout, $\bullet$). Note the fourfold difference in scale between *A* and *B*. *C*, Current-voltage relationship of peak Ca^{2+} currents obtained under each of these conditions. Both high-threshold and T-type Ca^{2+} currents are blocked by Mn^{2+} . Recovery of the T-type Ca^{2+} current is virtually complete following washout, whereas the high-threshold Ca^{2+} currents do not fully recover.

verapamil concentrations of 50 μM and 150 μM , the maximum high-threshold Ca^{2+} current was depressed by $65 \pm 5\%$ ($n = 4$) and $93 \pm 3\%$ ($n = 4$), respectively. In three of three neurons, verapamil also slightly decreased T-type Ca^{2+} currents as had been reported previously (Boll and Lux, 1985). Ethosuximide, which blocks T-type Ca^{2+} currents in thalamic neurons (Coulter et al., 1989a), did not appreciably block T-type Ca^{2+} currents in neostriatal neurons at concentrations of 350 μM or 700 μM ($n = 4$; data not shown).

Dihydropyridines such as nimodipine modulate "L-type" Ca channels (Nowycky et al., 1985; McCleskey et al., 1987; Aosaki and Kasai, 1989), which correspond in their kinetics to the current we refer to here as HVA_S . Therefore, we examined the effects of nimodipine on high-threshold Ca^{2+} currents in neostriatal neurons to determine whether the HVA_S currents in these cells correspond pharmacologically to "L-type" Ca^{2+} currents described in other neurons (Fox et al., 1987). In the majority

Figure 8. Effect of Cd^{2+} on high-threshold and T-type Ca^{2+} currents. *A*, Representative high-threshold Ca^{2+} current recordings obtained by stepping from a holding potential of -100 mV to the test potential of -10 mV in the absence ($\circ$) and presence ($\blacklozenge$) of Cd^{2+} (25 μM). *B*, The T-type Ca^{2+} current (inactivating current) is shown at the test potential of -40 mV in the absence ($\triangle$) and presence ($\bullet$) of 25 μM Cd^{2+} . The inactivating current was isolated as described in the Figure 3 caption. Note the difference in scale between *A* and *B*. The broken line indicates 0 current. *C*, Current-voltage relationships of peak Ca^{2+} currents from the same cell as in *A* and *B*. Cd^{2+} (25 μM) markedly diminishes the high-threshold Ca^{2+} currents while leaving the T-type Ca^{2+} current virtually unaltered.

of neurons (five of seven), nimodipine (10 μM) did not affect high- or low-threshold Ca^{2+} currents elicited from a holding potential of -100 mV, consistent with the reported voltage dependence of inhibition by dihydropyridines (Bean, 1984; Sanguinetti and Kass, 1984). When neurons were held at -40 mV and the HVA_S current was evoked in isolation with a step to -10 mV every 10 sec, nimodipine had a small blocking effect on this current (Fig. 10*A,B*), decreasing it by $17 \pm 5\%$ (mean $\pm$ SEM) in five of six neurons tested. Thus, only a small component of the HVA_S observed in these neurons was sensitive to blockade by nimodipine.

The dihydropyridine-insensitive, ω -conotoxin-sensitive component of the high-threshold Ca^{2+} current has been called the "N-type" Ca channel by Tsien and colleagues (Tsien et al., 1988). The ω -conotoxin sensitivity of neostriatal high-threshold

Figure 9. Effect of verapamil on high-threshold and T-type Ca^{2+} currents. *A*, Representative high-threshold Ca^{2+} current recordings obtained by stepping from a holding potential of -100 mV to the test potential of -10 mV in the absence ($\circ$) and presence ($\blacklozenge$) of verapamil ($50 \mu\text{M}$). *B*, The T-type Ca^{2+} current (inactivating current) is shown at the test potential of -40 mV in the absence ($\bullet$) and presence ($\triangle$) of $50 \mu\text{M}$ verapamil. The inactivating current was isolated as described in the Figure 3 caption. Note the difference in scale between *A* and *B*. The broken line indicates 0 current. *C*, Current–voltage relationships of peak Ca^{2+} currents from the same cell as in *A* and *B*. Verapamil ($50 \mu\text{M}$) blocks high-threshold Ca^{2+} currents while T-type Ca^{2+} currents are decreased by about 40%. Note the prominent inactivating component (HVA_i) of the high-threshold Ca^{2+} current in this cell.

Ca^{2+} currents evoked from two different holding potentials was determined. When neurons were held at -100 mV and combined HVA_s and HVA_i Ca^{2+} currents were evoked by a step to -10 mV, ω -conotoxin decreased the peak current by $29 \pm 5\%$ (mean $\pm$ SEM, three of four neurons; Fig. 10*C,D*). When the HVA_s Ca^{2+} current was isolated by holding the neuron at -40 mV and stepping to -10 mV, ω -conotoxin still blocked HVA_s Ca^{2+} current by $39 \pm 8\%$ (mean $\pm$ SEM, three of three neurons; Fig. 10*A,B*), indicating that the inhibitory action of ω -conotoxin in neostriatal neurons was not selective for the HVA_i current.

Localization of Ca^{2+} currents to the soma or dendrites of neostriatal neurons

The spatial distribution of Ca channels on the cell surface may be critically important for the processing and transmission of

electrical signals in neurons (Llinas, 1988). To determine if there is a differential distribution of high-threshold and low-threshold Ca channels on the soma versus the proximal dendrites of neostriatal neurons, we observed the changes in $[\text{Ca}^{2+}]_i$, as determined by fura-2 fluorescence imaging in the soma and proximal dendrites of neurons acutely isolated from neostriatum. Distal dendrites were not present following the acute isolation procedure. Neurons were depolarized in the presence and absence of $25 \mu\text{M}$ Cd^{2+} to block the high-threshold Ca^{2+} conductances, while leaving low-threshold Ca^{2+} conductances relatively intact. Depolarization of neostriatal neurons with 50 mM KCl resulted in an increase in $[\text{Ca}^{2+}]_i$ in both the soma and dendrites of these neurons (Figs. 11, 12). This $[\text{Ca}^{2+}]_i$ increase was reversible upon washout of the 50 mM KCl-containing solution. Application of Cd^{2+} alone did not change the baseline fluorescence ratios. A second 2 min KCl depolarization in the presence of $25 \mu\text{M}$ Cd^{2+} resulted in a markedly smaller increase in Ca^{2+} in both the soma and dendrites, suggesting that high-threshold Ca channels are present in both the soma and proximal dendrites of neostriatal neurons. The remaining Ca^{2+} increase in the somata and dendrites could be due to the presence of T-type Ca^{2+} channels that are virtually unaffected by $25 \mu\text{M}$ Cd^{2+} (Fig. 8). Alternately, it could represent the small proportion (approximately 21%) of high-threshold Ca channels that are not affected by $25 \mu\text{M}$ Cd^{2+} . A third depolarization with 50 mM KCl following washout of Cd^{2+} resulted in a much greater increase in $[\text{Ca}^{2+}]_i$ than was seen in the presence of Cd^{2+} , illustrating the reversibility of the block. Some reduction of the response to a third consecutive depolarization was evident in most neurons. Figures 11 and 12 show the changes in $[\text{Ca}^{2+}]_i$ and the time course, respectively, from two separate neurons. Similar changes were seen in two other neurons.

Discussion

Kinetic separation of Ca^{2+} current subtypes and their distribution in the neostriatum

These data show that there are multiple types of Ca^{2+} currents in neostriatal neurons. T-type Ca^{2+} currents could be clearly separated from high-threshold Ca^{2+} currents according to kinetic criteria. Thus, similar to T-type Ca^{2+} currents from other brain regions (Yaari et al., 1987; Coulter et al., 1989b; Hernández-Cruz and Pape, 1989; Fraser and MacVicar, 1991; Mogul and Fox, 1991; Takahashi et al., 1991), neostriatal T-type Ca^{2+} currents were activated at relatively hyperpolarized potentials, inactivated within tens of milliseconds during a depolarizing pulse, and exhibited steady-state inactivation that was half-maximal at -88 mV. T-type Ca^{2+} currents were found in 40% of neostriatal neurons. When present, they represented a small fraction of the total Ca^{2+} current, being only 21%, 8%, and 9% as large as the high-threshold Ca^{2+} current in small-, medium-, and large-sized neostriatal neurons, respectively.

In the present study, high-threshold Ca^{2+} currents were divided into two subtypes according to the presence or absence of steady-state inactivation. Thus, Ca^{2+} currents that were unaffected by a change in holding potential from -100 mV to -60 mV were called HVA_s Ca^{2+} currents whereas those that inactivated following a similar change in holding potential were called HVA_i Ca^{2+} currents. However, this subdivision of high-threshold currents may not correlate with distinct Ca channel types because (1) HVA_i Ca^{2+} currents appeared to belong to a heterogeneous group in that they exhibited highly variable degrees of inactivation during a 150 msec depolarizing pulse (but

Figure 10. Time course of block of high-threshold Ca^{2+} currents by nimodipine and ω -conotoxin. **A**, Representative plot of Ca^{2+} current versus time illustrating the effect of nimodipine (*Nimod*) and ω -conotoxin (*ctx*) on HVA_s Ca^{2+} currents. The cell was depolarized to -10 mV every 10 sec from a holding potential of -40 mV. Superfusion with medium containing $10 \mu\text{M}$ nimodipine resulted in a slight decrease of the high-threshold Ca^{2+} current. The subsequent addition of $5 \mu\text{M}$ ω -conotoxin to the superfusing medium resulted in a much larger decrease in Ca^{2+} current. Horizontal bars indicate the time of drug application. The numbered arrows indicate traces illustrated in **B**. **B**, Current recordings from the experiment illustrated in **A**. Each trace is an average of four consecutive recordings with the first one corresponding to the numbered arrow. **C**, Plot of Ca^{2+} current versus time illustrating the effect of ω -conotoxin on combined HVA_s and HVA_f Ca^{2+} currents. The cell was depolarized to -10 mV every 10 sec from a holding potential of -100 mV and superfused with $5 \mu\text{M}$ ω -conotoxin at the time indicated by the horizontal bar. The numbered arrows indicate traces illustrated in **D**. **D**, Current recordings from the experiment illustrated in **C**. Each trace is an average of three consecutive recordings, with the first one corresponding to the numbered arrow. For illustration, the first 1 msec of the capacitance transient was omitted from the current recording.

see Plummer and Hess, 1991), and (2) pharmacological studies with nimodipine and ω -conotoxin did not support this subdivision of high-threshold Ca^{2+} currents (see below). Nonetheless, this subdivision provides information on the percentage of Ca^{2+} current that would be available for activation at a given membrane potential. Virtually all neostriatal neurons exhibited HVA_s Ca^{2+} currents, and 70% of neostriatal neurons exhibited HVA_f Ca^{2+} currents. However, the HVA_f Ca^{2+} current represented only 23% of the total high-threshold Ca^{2+} current. The resting membrane potential of neostriatal neurons *in vivo* has been found to fluctuate between -75 mV and -55 mV (Wilson and Groves, 1981; Wilson et al., 1983) with an average resting potential of -64 ± 4 mV reported in a recent *in vivo* study of 110 neostriatal neurons (Calabresi et al., 1990). Thus, it appears that, at the resting potential of neostriatal neurons *in vivo*, most of the high-threshold Ca^{2+} current in neostriatal neurons would be expected to be available for activation and could thus play a role in determining firing characteristics of these neurons.

Pharmacological separation of Ca^{2+} current subtypes in the neostriatum

T-type and high-threshold Ca^{2+} currents were blocked to a similar degree by low millimolar concentrations of Mn^{2+} and Co^{2+} . However, T-type Ca^{2+} currents could be distinguished phar-

macologically from high-threshold Ca^{2+} currents by their insensitivity to $25 \mu\text{M}$ Cd^{2+} . Moreover, T-type Ca^{2+} currents were less sensitive to the Ca channel blocker verapamil than were high-threshold Ca^{2+} currents. These differences in sensitivity of high- and low-threshold Ca^{2+} currents to Cd^{2+} (Fox et al., 1987; Narahashi et al., 1987; Akaike et al., 1989; Mogul and Fox, 1991) and verapamil (Boll and Lux, 1985; Fedulova et al., 1985) have been observed in other cell types. In addition, some sensitivity of low-threshold Ca^{2+} currents to verapamil has been reported previously (Boll and Lux, 1985; Akaike et al., 1989). The anticonvulsant ethosuximide, which blocks thalamic low-threshold Ca^{2+} currents (Coulter et al., 1989a), did not affect neostriatal T-type Ca^{2+} currents. Differences in sensitivity of low-threshold Ca^{2+} currents to ethosuximide (Thompson and Wong, 1991) and another T-type Ca^{2+} current antagonist, amiloride, have been observed previously (Tang et al., 1988; Carbone et al., 1990), and such discrepancies might be explained by the existence of multiple types of low-threshold Ca^{2+} channels (Carbone et al., 1990; Huguenard and Prince, 1991).

There is general agreement that dihydropyridines affect primarily the "L-type" Ca channels as described by Tsien and colleagues (Fox et al., 1987; McCleskey et al., 1987; Tsien et al., 1988; Aosaki and Kasai, 1989; but see Akaike et al., 1989). However, dihydropyridine-induced blockade of non-"L-type"

Figure 11. Fura-2 fluorescence images show a Cd²⁺-sensitive rise in [Ca²⁺]_i in the somata and processes of striatal neurons caused by depolarization. Computer-processed images are illustrated from a representative experiment. Changes in the F₃₄₀:F₃₈₀ ratio, corresponding to relative changes in [Ca²⁺]_i, are represented by changes in pixel color. Every 45 sec, fluorescence images (averages of 8 frames at 30 Hz) were taken at two gain settings on the image intensifier: one that allowed visualization of the soma and another higher gain setting that allowed visualization of the thinner, fainter processes (shown as *insets* superimposed on the image of the soma taken at the higher gain setting). Representative images from an experiment are shown before (*A*) and during (*B*) depolarization by 50 mM KCl, which resulted in a rise in [Ca²⁺]_i. After washout of KCl, the cell was superfused with 25 μ M Cd²⁺ (*C*), and then depolarized in the presence of Cd²⁺ (*D*). Note the marked decrease in Ca²⁺ entry in both the soma and processes in the presence of Cd²⁺. Recovery (*E*) and repeated exposure to 50 mM KCl (*F*) are also shown. The fura-2 stain image at 360 nm shown in *H* (in red and white) illustrates how, when the gain was set for visualization of the processes, the readings obtained for the soma (in red) were saturated. It also illustrates the area in the top left corner from which the *insets* were taken.

neuronal Ca channels (Akaike et al., 1989; Sayer et al., 1990) as well as the failure of dihydropyridines to block some high-threshold noninactivating Ca²⁺ currents in the CNS (Leonard et al., 1987; Llinás et al., 1989; Mogul and Fox, 1991; Regan et al., 1991) have recently been reported. Our results indicate that nimodipine had only minimal effects on high-threshold Ca²⁺ currents in neostriatal neurons even when Ca²⁺ currents were elicited from a depolarized holding potential (−40 mV) at which the antagonist is most effective (Bean, 1984; Sanguinetti and Kass, 1984) and at which inactivating (“N-type”) components of the high-threshold Ca²⁺ current would be eliminated. These observations suggest that classical “L-type” Ca channels carry only a small component of the Ca²⁺ current in neostriatal

neurons. This is consistent with observations that dihydropyridine-sensitive Ca channels contribute a much smaller fraction of the total Ca²⁺ currents in neurons (Bean, 1989; Plummer et al., 1989; Regan et al., 1991) than in cardiac or smooth muscle (reviewed by Bean, 1989).

ω -Conotoxin blocked 29% and 39% of high-threshold Ca²⁺ currents elicited from hyperpolarized (−100 mV) and depolarized (−40 mV) holding potentials, respectively. In the initial description of “N-type” Ca channels, steady-state inactivation and rapid inactivation during strong depolarizations were thought to be characteristic of this channel (Fox et al., 1987; Tsien et al., 1988). However, the observation that ω -conotoxin blocked what appeared, by kinetic criteria, to be “L-type” Ca channels

led to controversy regarding whether ω -conotoxin blocks only "N-type" Ca channels (Kasai et al., 1987) or both "N-type" and "L-type" Ca channels (McCleskey et al., 1987). More recent single-channel (Aosaki and Kasai, 1989; Plummer et al., 1989; Plummer and Hess, 1991) and whole-cell studies (Carbone et al., 1990) of Ca^{2+} currents strongly suggest that dihydropyridine-insensitive, ω -conotoxin-sensitive channels may contribute both inactivating and sustained components of high-threshold Ca^{2+} currents and that ω -conotoxin-sensitive currents can be elicited from depolarized holding potentials (reviewed by Sher and Clementi, 1991). It appears that "N-type" Ca channels cannot be distinguished from "L-type" Ca channels by their whole-cell kinetics (Swandulla et al., 1991). Indeed, it has been proposed that "N-type" Ca channels be redefined as those channels that are sensitive to ω -conotoxin (Aosaki and Kasai, 1989; Plummer et al., 1989; Carbone et al., 1990). According to this redefinition, about 30–40% of neostriatal high-threshold Ca^{2+} currents are carried through ω -conotoxin-sensitive "N-type" Ca channels.

In the present study, nimodipine and ω -conotoxin were applied at concentrations that have been shown to effect maximal inhibition of high-threshold Ca^{2+} currents in central neurons (Mogul and Fox, 1991; Penington et al., 1991; Regan et al., 1991). Nonetheless, a large proportion of the high-threshold Ca^{2+} current in neostriatal neurons is clearly insensitive to block by either of these antagonists. Components of Ca^{2+} currents that are insensitive to dihydropyridines and nimodipine have also been reported in other brain regions including neocortex, hippocampus, and cerebellum (Sayer et al., 1990; Mogul and Fox, 1991; Regan et al., 1991) and in *Xenopus* oocytes injected with rat brain mRNA (Leonard et al., 1987). In cerebellum the proportion of high-threshold Ca^{2+} current that is resistant to these blockers is particularly large (Regan et al., 1991), and it has been proposed that high-threshold Ca^{2+} currents in this region may be carried by a distinct Ca channel, the "P-channel" (Llinás et al., 1989), which is blocked by funnel-web spider toxin (Lin et al., 1990). It is possible that the nimodipine- and ω -conotoxin-insensitive high-threshold Ca^{2+} currents in the neostriatum are of the "P-channel" type. However, a recent immunohistochemical study using an antibody against "P-type" Ca channels suggests that the density of P-type channels is low in the striatum as compared other brain regions (Hillman et al., 1991). Alternatively, it is possible that the nimodipine- and ω -conotoxin-insensitive high-threshold Ca^{2+} currents represent other types of as-yet-unidentified Ca channel in brain (Snutch et al., 1990).

Localization of Ca^{2+} conductances to the soma or dendrites of neostriatal neurons

Fura-2 imaging experiments were conducted to localize changes in $[\text{Ca}^{2+}]_i$ in neostriatal neurons following depolarization. The results suggest that high-threshold Ca^{2+} channels are present on both the soma and proximal dendrites of neostriatal neurons and that there is no large nonuniformity of high-threshold Ca^{2+} conductances in the soma versus the proximal dendrites. This finding contrasts with similar experiments in the hippocampus in which dendritic-somatic $[\text{Ca}^{2+}]_i$ gradients were detected following a 3 min application of 50 mM K^+ to neurons acutely isolated from hippocampal CA1, with a greater rise in $[\text{Ca}^{2+}]_i$ in the dendrites than soma of these neurons (Wadman and Connor, 1992). Similarly, Regehr et al. (1989) have observed that the stimulation-induced fura-2 Ca^{2+} signal in the somata of hippocampal pyramidal neurons lags behind and is much smaller in amplitude than the Ca^{2+} signal in the proximal den-

Figure 12. Time course of changes in $[\text{Ca}^{2+}]_i$ in a striatal neuron in the presence and absence of 25 μM Cd^{2+} . Changes in the spatial average of the F_{340}/F_{380} fluorescence of the cell soma (O) and processes (●) are plotted against time. The effects of three consecutive depolarizations with 50 mM KCl (K^+) are shown. The second KCl application occurred in the presence of 25 μM Cd^{2+} , which markedly decreased Ca^{2+} influx in both the soma and dendrites of striatal neurons. This suggests that high-threshold Ca^{2+} currents are present in both the somata and dendrites of striatal neurons. Horizontal bars indicate the time of application of 50 mM KCl or 25 μM Cd^{2+} .

drites. Previous studies in the inferior olive (Llinas and Yarom, 1981b), thalamus (Jahnsen and Llinas, 1984), and hippocampus (Yaari et al., 1987) have all yielded the interpretation that high-threshold Ca^{2+} channels are primarily localized in dendrites whereas low-threshold Ca^{2+} channels are primarily localized in the cell soma. More recently, however, high-threshold and low-threshold Ca^{2+} conductances have been found on all parts of the surface membrane of cultured chick dorsal root ganglion cells (Gottmann et al., 1991). Moreover, antibodies to "L-type" Ca channels have shown that these channels are located on both the soma and proximal dendrites of hippocampal neurons, with the highest density found at the apical dendrites (Westenbroek et al., 1990). It is possible to reconcile the findings of Westenbroek et al. (1990) with the delayed and diminished Ca^{2+} entry into the somata of hippocampal neurons observed by Regehr et al. (1989) by recognizing that somata have a much smaller surface-to-volume ratio than dendrites. The observation that the Ca^{2+} entry into the somata of neostriatal neurons was neither delayed nor smaller than the Ca^{2+} entry into the proximal dendrites of neostriatal neurons suggests that the density of high-threshold Ca^{2+} channels on the somata of neostriatal neurons may be greater than the density of these channels on the somata of hippocampal neurons.

Physiological significance of Ca^{2+} currents in the neostriatum

The T-type Ca^{2+} current was present in only 40% of neostriatal neurons and was small in relation to the total high-threshold Ca^{2+} current. The observation that T-type Ca^{2+} currents are generally of small amplitude in neostriatal neurons is consistent with previous findings. Spontaneous bursting and oscillatory behavior typical of neurons with prominent T-type Ca^{2+} currents is absent in neostriatal neurons *in vitro* (Calabresi et al., 1987; Galarraga et al., 1989), and low levels of spontaneous activity have been reported in these neurons *in vivo* (Hull et al., 1970; Sugimori et al., 1978). However, a Ca^{2+} -dependent potential resembling a low-threshold Ca^{2+} spike has been observed

in neostriatal neurons in TTX- and TEA-containing medium (Calabresi et al., 1987). In the present study, small-sized neostriatal neurons had proportionately more T-type Ca^{2+} current than medium- and large-sized neostriatal neurons. This suggests that there may be a subpopulation of smaller medium spiny neurons in which the T-type Ca^{2+} current plays a greater role in determining cell firing properties. Interestingly, 33% of striatal neurons *in vivo* exhibit regular spontaneous depolarizing potentials (Calabresi et al., 1990). Although phasic synaptic inputs may be responsible for this bursting activity, it may also result from intrinsic membrane Ca^{2+} conductances in a subpopulation of neurons.

The T-type Ca^{2+} current could affect the response of neostriatal neurons to stimuli near the resting membrane potential. There is a significant degree of overlap between the activation and inactivation curves of the T-type Ca^{2+} current, which suggests that there may be a small steady influx of Ca^{2+} at some membrane potentials. On depolarization, the inactivation of this steady Ca^{2+} conductance could contribute to the so-called "anomalous rectification" in the depolarizing direction (increased input resistance during depolarizing current injection; Kita et al., 1985a). This is supported by the finding that "anomalous rectification" in the depolarizing direction is decreased in Ca^{2+} -free medium (Kita et al., 1985a).

High-threshold Ca^{2+} currents were much more prominent than the T-type Ca^{2+} current in neostriatal neurons. The importance of high-threshold Ca^{2+} currents in influencing the firing properties of these neurons has been suggested by current-clamp studies (Kita et al., 1985b; Misgeld et al., 1986; Calabresi et al., 1987, 1990; Cherubini and Lanfume, 1987; Galarraga et al., 1989). Blockade of Ca^{2+} currents blocks spontaneous depolarizing potentials and late depolarizations (Calabresi et al., 1990) and results in a decrease in the size of action potentials (Galarraga et al., 1989). Moreover, a Ca^{2+} -activated K^{+} conductance appears to underlie the afterhyperpolarizing potential in neostriatal neurons (Galarraga et al., 1989). Via this mechanism, Ca channel activity may profoundly affect the rate of firing of neostriatal neurons in response to depolarizing stimuli. One of the central functions attributed to Ca^{2+} currents is their critical role in neurotransmitter release. Both "N-type" and "L-type" Ca channels have been implicated in this process (reviewed by Bean, 1989), although it is likely that Ca^{2+} currents at the soma exhibit somewhat different properties from Ca^{2+} currents at the presynaptic terminals (Lemos and Nowycky, 1989; Stanley, 1991).

Voltage-dependent calcium conductances are an important site for neurotransmitter-mediated modulation of neuronal activity. A number of the major neurotransmitters in the neostriatum (dopamine, ACh, and glutamate) modulate Ca^{2+} currents in other regions (Toselli and Lux, 1989; Lester and Jahr, 1990; Williams et al., 1990; Chervenskaya et al., 1991; Lledo et al., 1992). Future studies may determine whether the Ca^{2+} currents in neostriatal neurons are the site of modulation of neostriatal firing properties by neurotransmitters or neuromodulators.

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