The activity of the striatum is regulated by glutamate and dopamine neurotransmission. Consequent to striatal dopamine depletion the corticostriatal excitatory input is increased, which in turn can raise intracellular calcium levels. We investigated changes in the neuronal expression of the calcium binding protein calretinin related to dopamine depletion and L-DOPA administration. Immunohistochemical methods were used to assess calretinin in the striatum of rats with unilateral lesions of the nigrostriatal system. In these animals we observed a loss of the patchy distribution of calretinin fibers. Moreover, after dopaminergic depletion we detected two new, not previously described, calretinin cell types, the presence of which could be related to morphological changes induced by loss of a dopaminergic input. We also found an increase in the number of calretinin-labeled cells in the striatum ipsilateral to the lesion compared to the contralateral striatum or to the striatum of normal rats. This increase was mostly evident at 3 weeks postlesion and tended to decrease toward normal levels at 6, 10, and 18 weeks postlesion. In unlesioned animals, L-DOPA administration did not induce changes in the expression of calretinin. In unilaterally lesioned animals, L-DOPA reversed the increase in the number of calretinin-positive cells induced by the lesion. However, chronic L-DOPA administration was less effective than acute L-DOPA in reversing the effect of the lesion. The present data suggests that striatal calretinin neurons are sensitive to dopamine depletion. Increased expression of calretinin in striatal cells may be consequent to enhanced striatal excitatory input.

Key Words: 6-OHDA lesion; striatum; interneurons; immunolabeling; calcium binding proteins; calretinin.

INTRODUCTION

In Parkinson’s disease (PD), the nigrostriatal system degenerates and the striatum is depleted from its dopaminergic input. As a result the striatum undergoes changes, including biochemical, physiological, as well as morphological (62, 46), that ultimately result in the symptomatology of PD. Recent findings have drawn a lot of attention to the role of glutamate and its interaction with dopamine (DA) in the pathology of PD (8, 57). The glutamatergic corticostriatal and the dopaminergic nigrostriatal afferents seem to converge on both striatal-projecting neurons and interneurons (40, 55, 56, 49, 23, 34, 32, 33, 18, 15, 52, 59). The corticostriatal glutamatergic system seems to exert a general excitatory effect (14, 13), while the dopaminergic system is believed to reduce the responses elicited by glutamatergic corticostriatal stimulation (14). DA is considered to exert an inhibitory effect mainly on striatal-pallidal GABA/enkephaline projection neurons (22, 45, 53), and possibly on striatal interneurons (34, 33, 18, 15, 52), whereas it either inhibits or enhances the activity of D1 containing striatal-nigral neurons depending on the level of membrane depolarization (25). In addition, the glutamatergic and dopaminergic afferent systems are functionally linked through pre- and postsynaptic interactions with a possible inhibitory role of DA on glutamatergic terminals (57, 14, 45, 53). Given these interactions, nigrostriatal cell degeneration not only removes a direct DA inhibitory input but, indirectly, enhances the excitatory input to the striatum, thus increasing the excitability of distinct populations of striatal neurons (13, 45, 43). Increased glutamatergic activity can induce abnormal increases in cellular calcium concentration, which in turn can interfere with neuronal functioning or even cause striatal neurotoxicity (44, 42, 17, 19). However, only few studies reported loss of striatal neurons after DA deafferentation (11, 9, 44). Others have reported structural and synaptic reorganization of striatal output neurons as well as intrinsic neurons, but not cell degeneration (46, 3, 28). There is evidence that distinct populations of striatal...
neurons respond differently to increased glutamatergic receptor activation, and that this difference may be related to the presence of calcium binding proteins (60). Therefore, it is possible that, after DA depletion, subpopulations of striatal cells are able to withstand excessive excitatory inputs because of their ability to maintain calcium homeostasis.

Calcium binding proteins such as calbindin, parvalbumin, and calretinin are very good candidates to test this hypothesis. These proteins are believed to be involved in the regulation of intracellular calcium concentrations (20, 4, 24) and their expression has been shown to change following neuronal excitation (36) or altered neuronal functioning (37, 27). Of these calcium binding proteins, calretinin may provide some insight on the consequences of striatal DA depletion. For instance, in the rat striatum, calretinin is expressed in the patches compartment (22, 26) in a network of fibers of extrastriatal origin, one component of which has been suggested to be of dopaminergic origin (30, 5, 29). Calretinin is also expressed in a small population of aspiny GABAergic interneurons (20, 5), which has been suggested to be in synaptic contact with DA afferents as well as with glutamatergic corticostriatal afferents (5, 6). The postsynaptic target of these neurons remains, however, unidentified (31). Both striatal calretinin-positive fibers and neurons can be influenced by degeneration of the nigrostriatal system. Lesion of the nigrostriatal pathway is expected to disrupt the patchy organization of calretinin fibers. As for those striatal neurons that can express calcium-binding proteins, such as calretinin, the question is whether striatal DA depletion induces increased calretinin cell expression.

Administration of the DA precursor L-DOPA is the therapeutic approach used to reinstate striatal dopaminergic function. L-DOPA may reverse the increased excitatory effects induced by DA depletion. This hypothesis is consistent with the finding showing a decrease in the activity of corticostriatal glutamatergic terminals after administration of DA agonists (21). However, this is apparently in contrast with the observation that after repeated treatment L-DOPA may lose its antie excitatory effect (35). It has also been shown that exposure of cultured rat striatal cells to L-DOPA induces neurotoxicity possibly mediated by release of endogenous glutamate (38, 16). This neurotoxicity was prevented by glutamate receptor antagonists and attenuated by calcium removal, suggesting the involvement of glutamate receptor stimulation in the neurotoxic effects of L-DOPA (7, 47). Hence, the question arises whether chronically given L-DOPA reduces or rather increases the expression of calretinin in the DA depleted striatum.

To address these questions we studied the expression of calretinin (2) after DA depletion and L-DOPA administration. We used the well known 6-hydroxydopamine (6-OHDA) rat model of Parkinson's disease. Animals carrying unilateral lesions, were treated with L-DOPA or saline either acutely at different time points after the lesion or chronically for different periods of time for up to 16 weeks. We assessed calretinin neuronal expression in the striatum of these rats using standard immunohistochemical methods.

**MATERIALS AND METHODS**

**Unilateral 6-OHDA Lesions**

Thirty minutes before surgery, 63 male Wistar rats [250–300 g, Zur: WIST (HanIbm), Institute of Toxicology, Schwerzenbach, CH] were given pargyline (40 mg/kg, i.p.) to prevent the metabolism of 6-OHDA. Animals were then anesthetized with pentobarbital (Nembutal 50 mg/kg, i.p.) and placed in a stereotaxic frame. A hole was drilled in the skull to deliver 6-OHDA unilaterally at the level of the medial forebrain bundle (MFB): 4 mm posterior to Bregma, 2 mm to the right of the midline, and 8 mm ventral to the surface of the cortex. 6-OHDA (8 μg in 2 μl) was injected into the MFB through a 28-gauge stainless steel cannula connected by a teflon tube to a 10-μl Hamilton syringe. The injection speed was μl/min, and the cannula was left in place for an additional 3–5 min postinjection. After the lesion, animals were allowed to recover for 2 weeks.

**L-DOPA Treatment**

Two weeks after the lesion, four groups of lesioned animals were treated daily with benserazide (25 mg/kg i.p.), followed 30 min later with L-DOPA (50 mg/kg i.p.), for 1, 4, 8, or 16 weeks (n = 16; 4 per group). A control group was treated with saline (n = 8). The treatment terminated 3, 6, 10, or 18 weeks after the lesion. Two additional groups of lesioned rats were treated only at 3, 6, 10, or 18 weeks postlesion, with a single injection of L-DOPA (50 mg/kg i.p., n = 16; 4 per group) or saline (n = 12; 3 per group). A control group of uninjured rats was either untreated (n = 3) or treated with a single injection of L-DOPA (n = 4) or saline (n = 4) before sacrifice. All drugs were purchased from Sigma (Buchs, Switzerland). Animal care procedures and experimental protocols were approved by the Ethics Committee of the Veterinary Office of the Canton of Zurich, Switzerland, and were in accordance with NIH guidelines.

**Immunohistochemistry**

Two hours after the last L-DOPA or saline injection, rats were deeply anesthetized with Nembutal (100 mg/kg, i.p.) and then perfused through the ascending aorta at a flow rate of 40 ml/min. A perfusion with 70 ml of 0.9% saline was followed by a cold solution of 4% para-
formaldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.4, 250 ml after clamping the descending aorta). Brains were removed and postfixed for 1–2 h in cold fixative and then transferred to a 30% sucrose solution overnight at 4°C. Brains were cut with a freezing microtome, and transverse sections (40 μm thick) were collected throughout the rostrocaudal extent of the striatum and substantia nigra. Using standard ABC methods, sections were processed for tyrosine hydroxylase (TH) and calretinin immunolabeling. Following 3 × 5-min rinses in 0.1 M PBS (pH 7.4), free floating sections were blocked for 1 h in PBS containing 5% normal goat or donkey serum plus 0.3% Triton X-100. Sections were then incubated in a solution of PBS and 2% normal goat or donkey serum plus 0.15% Triton X-100, containing the primary antibodies sheep anti-TH (1:1000, Pel-Freez, Roger, Arkansas), or rabbit anti-calretinin, (1:4000, Chemicon, Lucerne, CH), overnight at 4°C. Sections were rinsed and incubated for 1 h in biotinylated secondary antibodies (donkey anti-sheep and goat anti-rabbit, 1:300, Jackson Immuno Research, West Grove, PA), in a solution of 2% goat or donkey serum plus 0.15% Triton X-100, at room temperature. Subsequently, sections were treated with ABC complex (Vectastain Elite, Vector Laboratories, Burlingame, CA) for 1 h at room temperature followed by 3 × 5-min rinses in 0.1 M Tris buffer (TB, pH 7.4). Immunoreactivity was visualized with 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma, Buchs, CH) and 0.004% H2O2 in TB for 3–5 min. Nickel chloride (0.08%) was added to the DAB solution to intensify the staining. Sections were then rinsed in TB, mounted on slides, dried, and coverslipped. Controls for nonspecific staining were performed in which either the first or secondary antibody was omitted. These controls did not produce any staining.

Quantification and Analysis

A total of four sections/brain taken at a rostral level of the striatum, between AP 1.60 and 1.00 from Bregma, and four sections between AP –4.52 and –6.30 for the substantia nigra (Paxinos and Watson, 1986), were analyzed for each marker. Labeled sections for TH and calretinin were examined using bright-field microscopy. TH is a marker for dopaminergic cells and fibers and therefore it was used to estimate the extension of the lesions. Striatal calretinin labeled cells from the lesioned side and from the nonlesioned side were counted manually using a Zeiss Axioplan microscope (Germany) equipped with an eyepiece with a grid. For quantification purposes, cells were considered in which the calretinin reaction product could be unequivocally identified in the soma independently of the intensity of the labeling. No distinction was made between the size and the shape of the cells. The measurement of the maximum diameter of stained cell profiles (n = 50), within each section, were obtained using a Zeiss Axioshot microscope (Germany) in combination with a Kodak Megaplus video-camera (Eastman Kodak, San Diego, CA) and a computer image analysis program Image-Pro Plus (Media Cybernetics, Silver Spring, MD). Digitized bright-field images were captured using the system described above. For statistical evaluations we used an analysis of variance (ANOVA) and the post-hoc Fisher’s PLSD test. A P value ≤0.05 was considered to represent a significant difference. Results are given as mean ± SEM.

RESULTS

Effect of the Lesion: Loss of Tyrosine Hydroxylase Immunolabeling

Administration of 6-OHDA induced extensive dopaminergic cell loss in all animals. Dopaminergic nigrostriatal cells were identified by their immunoreactivity for TH, and as shown in Fig. 1B, a dramatic loss of TH-labeled cells and fibers was observed in the le-
sioned substantia nigra pars compacta (SNc). A complete loss of dopaminergic fibers was consistently observed in the lesioned striatum as demonstrated by the absence of TH labeling (Fig. 1A). The ventral tegmental area, the nucleus accumbens, and the olfactory tuberculum were also affected by the lesion (Fig. 1A).

Calretinin Neuropil Labeling in the Striatum of 6-OHDA-Lesioned Rats

The calretinin neuropil labeling in the striatum of unlesioned rats and in the contralateral striatum of lesioned rats treated with saline did not differ. Therefore, only the contralateral and ipsilateral striatum of lesioned animals will be described and illustrated. All sections analyzed showed immunoreactivity for calretinin in striatal fibers and in a population of striatal cells. In the side contralateral to the lesion, the striatal neuropil labeling showed a gradient being more intensely labeled in the medial and ventral regions compared to more lateral regions (Fig. 2A). In the most dorsomedial and ventral regions of the striatum, calretinin fibers were distributed in patches as shown in Fig. 2B. After the lesion, a general decrease in the intensity of calretinin neuropil labeling and a clear loss of the patchy distribution pattern was observed in the lesioned side of the striatum as compared to the nonlesioned side (Figs. 2A and 2C). This was particularly evident in the most medial regions where the patchy distribution, observed in the nonlesioned side, was absent (arrows in Figs. 2B and 2C).

Calretinin-Labeled Cells: Distribution and Morphology in the Striatum of 6-OHDA-Lesioned Rats

The distribution and morphology of striatal calretinin-positive cells in unlesioned rats and in the contralateral striatum of lesioned rats treated with saline was similar. Therefore, only calretinin-labeled cells in
the contralateral and ipsilateral striatum of lesioned rats will be described and illustrated. In the striatal side ipsilateral and contralateral to the lesion, and at each time point postlesion, calretinin was expressed in a heterogeneous population of aspiny neurons varying from small (7–10 μm), medium (10–15 μm), to large soma (15–25 μm) (Figs. 3A–3C). Most labeled cells had round, ovoid, or fusiform soma and were unipolar and bipolar although multipolar cells were not infrequently observed (Fig. 3C). One, two, and sometimes three or more primary dendrites originated near the soma giving rise to several secondary dendrites that could be followed for more than 200 μm. Most cells were intensely labeled and very frequently showed beaded dendrites (Fig. 3). 

In the striatum of lesioned animals, we also observed two not previously described types of calretinin positive cells. The first cell type was often observed in the most dorsomedial areas of the striatum, just beneath the corpus callosum (Fig. 4A). As other calretinin-labeled cells, they were small (7–10 μm) with a round soma and mainly unipolar. However, the dendrites of these cells branched very near to the cell body in a bush-like pattern, showing a very irregular aspect (curly like) which appeared different from the typical beaded or smooth dendrites of most calretinin cells described here (Fig. 4B). These cells were observed both in the ipsilateral and contralateral striatal sides of lesioned rats at each time point postlesion. The second unreported type of calretinin-labeled cell was identified essentially in the striatal side ipsilateral to the lesion. It was located in the same striatal regions, but it was much rarer than the previous type. These cells had a round (12–15 μm) unipolar or bipolar soma. The primary dendrite gave rise to few branches that had the peculiarity of possessing a small number of tiny widely interspaced spine-like structures (Fig. 5). They were mainly, but not only, observed in animals sacrificed 18 weeks postlesion.

Quantitative Analysis of Calretinin-Positive Cells in the Striatum of Unlesioned Animals Either Untreated or Treated Acutely with L-DOPA or Saline

The number of calretinin positive cells, in both striatal sides, of nonlesioned untreated control rats ranged between 45–60 cells/section. To identify the effect of an acute L-DOPA treatment, two additional groups of nonlesioned animals received a single injection of L-DOPA or saline. An ANOVA revealed that in these groups of nonlesioned animals, both striatal sides had a similar number of calretinin labeled cells (F(1, 8) = 2.6, P = 0.15). The number of cells on either side was not affected by L-DOPA or saline treatments as shown by a nonsignificant Treatment effect (F(2, 8) = 1.1, P = 0.39) (Fig. 6). Therefore, these groups of animals were combined to form a single unlesioned control group.

Quantitative Analysis of Striatal Calretinin-Positive Cells after 6-OHDA Unilateral Lesions

To estimate the impact of the lesion per se on the number of calretinin-labeled cells in both striatal sides, an ANOVA was performed comparing the above mentioned unlesioned control group to the four groups of unilaterally lesioned animals treated with a single injection of saline. The four groups consisted of animals sacrificed at four different time points postlesion (3, 6, 10, and 18 weeks). The analysis showed that the ipsilateral striatum had a higher number of calretinin-labeled cells compared to the contralateral striatum (F(1, 18) = 78.5, P < 0.001). A significant difference between groups (F(4, 18) = 3.19, P < 0.04) and a Fisher’s post hoc test revealed that the number of calretinin cells, in both striatal sides, was significantly higher at week 3 postlesion when compared to the controls (P < 0.003), or week 6 (P < 0.03), or week 10 (P < 0.02), or week 18 (P < 0.02). The latter three lesioned groups did not differ from controls or from each other (Figs. 7A and 7B).

Quantitative Analysis of Striatal Calretinin-Positive Cells after L-DOPA Given Chronically or Acutely

We evaluated the effects of L-DOPA, given either chronically or acutely, on the number of calretinin-positive cells in the striatum of lesioned animals. The analysis showed that the number of calretinin cells was higher in the ipsilateral side compared with the contralateral side of the striatum (F(1, 32) = 121.9, P < 0.0001). In order to evaluate the effects of Time postlesion (weeks 3, 6, 10, 18) and Treatment (L-DOPA acute or chronic, or saline), we performed an analysis on the contralateral and ipsilateral side separately (Figs. 7A and 7B). The analysis for the contralateral side showed a significant effect of Time post-lesion (F(3, 22) = 4.39, P < 0.01), reflecting a higher number of cells at week 3 compared to week 6 (P < 0.008) and week 10 (P < 0.003), and a trend for week 18 (P < 0.07), (Fig. 7A). The Treatment effect reached a borderline level of significance (F(2, 22) = 3.18, P < 0.06), which was indicative of a reduction in the number of labeled cells after L-DOPA, (Fig. 7A). The analysis for the ipsilateral side revealed a significant effect of Time (F(2, 32) = 6.41, P < 0.002), revealing that the increase of calretinin-labeled cells was higher at week 3 compared to week 6 (P < 0.002), week 10 (P < 0.002), and week 18 (P < 0.002) (Fig. 7B). A highly significant effect of Treatment (F(2, 32) = 9.69, P < 0.005) (see inset in Fig. 7) showed that after acute L-DOPA the number of calretinin-labeled cells was significantly lower than after chronic L-DOPA (P < 0.04), which in turn was significantly lower than after saline (P < 0.03).
DISCUSSION

After DA depletion the striatum undergoes structural and functional changes, some of which can be revealed evaluating the expression of the calcium binding protein calretinin. First of all, our light microscopic observations showed that nigrostriatal fibers contribute heavily to the patchy distribution of calretinin fibers in the intact striatum, especially in its most medial and ventral regions (26). As a result of a nigrostriatal dopaminergic lesion, there is an almost complete loss of calretinin fibers and consequently of their patchy distribution. Thus, calretinin expression is useful to identify and visualize alterations of the striatal patches compartment in degenerative disorders such as PD.

Our observations on the morphology and distribution of striatal calretinin cells generally agreed with previous descriptions, showing that calretinin is expressed in a small population of cells with a variable size and shape (20, 5). However, we also observed two not previously described cell types in the rat striatum. The first cell type was characterized by a highly branched dendritic tree, with dendrites showing an irregular type of pattern (curly-like). These cells were observed in both striatal sides of lesioned rats, at each time postlesion, but not in the striatum of normal animals. It is possible that, as a consequence of DA denervation, some calretinin expressing neurons underwent structural changes, such as increased dendritic arborization (46). In addition, we report a striatal calretinin cell type characterized by the presence of spine-like structures on the dendritic shaft. Although the presence of spines has not been reported in striatal calretinin cells in rodents, calretinin neurons with spiny dendrites have very recently been shown in the human striatum (51). In our case, these cells were observed only in the lesioned side of the striatum, and mainly, but not only, in animals sacrificed 18 weeks after the lesion. This could relate their presence to processes of reorganization induced by the lesion (46). Further investigations at the electron microscopic level should reveal whether these calretinin cells are, for instance, medium spiny neurons with regressed spines (46, 28) or if they are part of a distinct cell type. Altogether, these findings may suggest that the presence of calretinin is not restricted to striatal interneurons but that under certain circumstances calretinin can also be expressed in certain projection neurons.

Very importantly, we have shown that the population of striatal cells expressing calretinin increases after degeneration of the nigrostriatal dopaminergic neurons. This increase in calretinin expression is associated with the appearance of new morphological cell types, such as those described in this study. Our findings suggest that calretinin is not only a general indicator of neurodegeneration, but also a marker for distinguishing between different stages of degeneration and for identifying specific cellular changes associated with the disease.

FIG. 3. Calretinin-labeled cells in the nonlesioned (A) and in the lesioned striatum (B, C). In A, B, and C, the arrows indicate dendrites with a typical beaded labeling pattern. In the nonlesioned side (A) the arrowheads indicate a patch of calretinin fibers. Calibration bar, 50 µm.
This effect was observed in both striatal sides (39) but was significantly more pronounced in the side ipsilateral to the lesion. This increase could be related to changes of the DA/glutamate balance (8, 3). After DA depletion, the glutamatergic effect is enhanced (43, 12) and the excitatory/inhibitory balance shifts toward an excessive excitatory input. Although little is known regarding the types of receptor on the striatal calretinin cells, there is anatomical evidence that these cells interact with glutamatergic excitatory afferents of cortical origin (30, 5), as well as with dopaminergic, possibly inhibitory, nigrostriatal afferents (45, 6). Therefore, we suggest that after DA depletion some populations of striatal cells, including calretinin cells, are subjected to an increased excitatory input that can in turn increase their cellular calcium levels (60). This can lead to amplified calretinin expression in more cells than under normal conditions. It has consistently been shown that the calcium buffering capacity of neurons can be dynamically regulated at the level of gene transcription in response to prolonged stimulation (36, 61). From our study, it is, however, not possible to establish whether these processes are specifically restricted to striatal calretinin interneurons or whether additional striatal populations are involved, as perhaps suggested by the presence of calretinin in cells with spiny dendrites. Furthermore, our results indicate that this increase in calretinin cell expression is transitory. As the time postlesion increases, the number of calretinin-labeled cells in both striatal sides goes back to normal levels perhaps indicating that increased calretinin expression is part of compensatory mechanisms taking place after the lesion (62, 54).

Our results show that in unlesioned animals acute L-DOPA did not change calretinin cell expression. Exogenous L-DOPA given for short periods of time to intact animals does not alter normal striatal DA levels (41). Therefore, no evident changes should be expected in the expression of calretinin in striatal neurons of normal animals. However, in lesioned animals a single L-DOPA challenge given at 3 weeks postlesion reversed the effect of the lesion by reducing the number of cal-

**FIG. 4.** Photomicrographs illustrating a distinctive type of calretinin positive cell found in the most mediodorsal areas of the nonlesioned and lesioned striatum (arrows, A). Note that the dendritic tree has a very irregular aspect and that it branches very near to the cell body in a bush-like pattern. The arrows in (B) point to one of these cell's dendrites at higher magnification. Note the difference in these cell's morphology when compared to a more common type of calretinin positive cell (arrowhead). Calibration bar, 50 μm.
Crecibly, in DA-depleted animals, a single administration of L-DOPA may be sufficient to reinstate normal levels of excitation (21), therefore reducing the increase in calretinin expression as well. Interestingly, a single injection of L-DOPA leads to a stronger decrease in calretinin expression than a chronic treatment. The mechanisms underlying this effect still need to be clarified. It is possible, however, that chronic L-DOPA treatment leads to desensitization of DA receptors (58, 50) or reduces the capacity of the striatum to produce enough active DA (10) to counteract increased excitatory effects. Thus, our results may be suggestive of a reduced effectiveness of repeated L-DOPA administration in opposing the effects of increased excitatory neurotransmission. This may also have interesting implications concerning the question of whether L-DOPA exerts toxic effects on striatal cells (1). Under our experimental conditions, chronic L-DOPA did not decrease the number of calretinin-labeled cells below normal levels, possibly excluding neurotoxic effects. However, it cannot be excluded that after longer periods of administration L-DOPA fails to normalize the excitatory/inhibitory balance. Indeed it has been suggested that repeated L-DOPA treatment may eventually facilitate glutamate-induced toxicity (42, 44).

In summary, our results suggest that the expression of calretinin in striatal cells is sensitive to changes related to striatal DA depletion and a consequent shift

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**FIG. 5.** Photomicrograph illustrating a rare calretinin positive cell found in the lesioned striatum. Note that the soma gives rise to few branches that possess a small number of tiny largely interspaced spine-like structures (arrows). The asterisks show these spine-like structures attached to fibers. Calibration bar, 50 μm.

**FIG. 6.** The number of calretinin cells (mean ± SEM) in the striatum of unlesioned untreated animals (n = 3) and after acute L-DOPA (n = 4) or saline (n = 4). The number of calretinin-positive cells does not change in the three treatment conditions (P = 0.4) or in the right vs the left side of the striatum (P = 0.15).
in the striatal excitatory/inhibitory balance toward enhanced excitation. The increase in the number of neurons expressing calretinin may be part of the compensatory mechanisms that take place after DA depletion. This is for instance indicated by the reversal of this increase by extended periods of time after the lesion. The present data also showed that acute L-DOPA reverses the effect of the lesion as it effectively reduced the number of calretinin-positive cells. This may be indicative of a process of normalization of the excitatory/inhibitory balance due to the newly produced DA from exogenous L-DOPA. The significance of this result should, however, be further investigated since (a) the effect of L-DOPA is reduced after chronic treatment, which could have interesting implications for the therapeutic approach to PD, and (b) these effects were demonstrated in a small population of striatal neurons and their relevance to the overall functioning of the striatum requires further studies.

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