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# Dopaminergic modulation of early signs of excitotoxicity in visualized rat neostriatal neurons

Carlos Cepeda, Christopher S. Colwell, Jason N. Itri, Ehud Gruen and Michael S. Levine
Mental Retardation Research Center, University of California, Los Angeles, Los Angeles, CA 90024-1759, USA

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## Abstract

Cell swelling induced by activation of excitatory amino acid receptors is presumably the first step in a toxic cascade that may ultimately lead to cell death. Previously we showed that bath application of *N*-methyl-D-aspartate (NMDA) or kainate (KA) produces swelling of neostriatal cells. The present experiments examined modulation of NMDA and KA-induced cell swelling by dopamine (DA) and its receptor agonists. Nomarski optics and infra-red videomicroscopy were utilized to visualize neostriatal medium-sized neurons in thick slices from rat pups (12–18 postnatal days). Increase in somatic cross-sectional area served as the indicator of swelling induced by bath application of glutamate receptor agonists. NMDA induced cell swelling in a dose-dependent manner. Activation of DA receptors in the absence of NMDA did not produce swelling. DA and the $D_1$ receptor agonist SKF 38393, increased the magnitude of swelling produced by NMDA. This effect was reduced in the presence of the $D_1$ receptor antagonist, SCH 23390. In contrast, activation of $D_2$ receptors by quinpirole decreased the magnitude of NMDA-induced cell swelling. DA slightly attenuated cell swelling induced by activation of KA receptors. Quinpirole produced a significant concentration-dependent reduction in KA-induced swelling while SKF38393 increased KA-induced swelling, but only at a low concentration of KA. Together, these results provide additional support for the hypothesis that the direction of DA modulation depends on the glutamate receptor subtype, as well as the DA receptor subtype activated. One possible consequence of these observations is that endogenous DA may be an important contributing factor in the mechanisms of cell death in Huntington's disease.

## Introduction

Degeneration of neostriatal (NS) medium-sized spiny neurons is the major neuropathological sequela of Huntington's disease. According to the excitotoxic hypothesis, excessive release of glutamate or altered sensitivity of glutamate receptors are factors predisposing to cell death in this disorder (for a review see DiFiglia, 1990). The *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors has been the most frequently examined with respect to this excitotoxic role in the NS because it reproduces more closely the cellular damage observed in Huntington's disease (Schwarcz *et al*., 1983).

Although the glutamatergic excitotoxic hypothesis has received considerable support, the roles of other neurotransmitter systems in the NS also have been examined. Recent evidence has suggested that dopamine (DA), as well as its precursor L-DOPA or its metabolites, may function either to induce or modulate excitotoxicity (Olney, 1990; Filloux & Townsend, 1993). For example, DA-induced neurotoxicity can occur under conditions of increased DA availability and decreased antioxidant capacity (Zigmond & Hastings, 1998) and activation of $D_1$ receptors may induce neuropathological damage in the NS (Kelley *et al*., 1990). Furthermore, D-amphetamine, an agent that induces DA release and blocks reuptake causes axonal and neuronal degeneration in the NS and the dorsal agranular cortex (Ryan *et al*., 1990).

There is considerable evidence demonstrating that DA modulates neurotoxicity in the NS as well as in other neural areas. DA protects cultured NS neurons against kainate (KA) receptor mediated cytotoxicity (Amano *et al*., 1994). Destruction of the nigrostriatal pathway protects against neurotoxicity induced by cerebral ischaemia (Globus *et al*., 1987, 1988) and reduces the damage produced by excitatory amino acids (EAAs) in the NS (Chapman *et al*., 1989; Buisson *et al*., 1991). Blockade of DA receptors prevents damage to dopaminergic terminals by metamphetamine (Marshall *et al*., 1993; Ohmori *et al*., 1993). In retinal neuronal cell cultures, DA protects against NMDA receptor-mediated glutamate toxicity via $D_1$ receptors (Kashii *et al*., 1994). In cultured rat forebrain neurons, DA and glutamate interact to potentiate cell death, possibly via oxidative stress (Hoyt *et al*., 1997).

In view of this body of evidence, it becomes important to evaluate how DA and glutamate receptors interact in models of excitoxicity. One way to examine this interaction is to analyse cellular morphological changes that occur after exposure to EAAs and DA. Previous studies using on-line infra-red videomicroscopy in cerebral cortical slices showed that increases in somatic area (cell swelling) reflect early signs of neurotoxicity (Dodt & Zieglgänsberger, 1990, 1994; Dodt *et al*., 1993). In cell cultures, it is established that continuous

*Correspondence*: Michael S. Levine, as above. E-mail: mlevine@mednet.ucla.edu

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activation of EAA receptors can induce toxicity that ultimately leads to cell death (Choi, 1988; Rothman, 1992). A first step in this toxic cascade is the induction of cell swelling, an event that occurs within minutes after exposure to high concentrations of EAA receptor agonists. Previously, we have examined this phenomenon in visualized NS cells in thick slices and have described a number of its properties, potential ionic mechanisms, and modulation by activation of metabotropic glutamate receptors (Colwell & Levine, 1996; Colwell *et al*., 1996). In the present study, we used EAA agonist-induced cell swelling as an index to evaluate the modulatory actions of DA and its specific receptor agonists.

## Materials and methods

All procedures were carried out in accordance with the USPHS, Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at UCLA. Sprague–Dawley rat pups (12–18 days old, $n = 68$) were used in these experiments. The choice of this age range was based on our experience with NMDA-induced swelling from visualized cells using infra-red videomicroscopy and differential interference contrast optics (IR-DIC) in NS slices (Cepeda *et al*., 1995, 1998; Colwell & Levine, 1996; Colwell *et al*., 1996). This age represents a compromise between the presence of NMDA responses, the effects of DA and the ability to visualize cells, which becomes more difficult in older tissue.

Our procedures for tissue preparation, visualization and measurement of cell cross-sectional areas have been described (Cepeda *et al*., 1995, 1998; Colwell & Levine, 1996; Colwell *et al*., 1996). Briefly, rats were anaesthetized with halothane and then decapitated. After dissection, brains were placed in cold oxygenated artificial cerebrospinal fluid (ACSF) (in mM: NaCl, 130; $NaHCO_3$, 26; KCl, 3; $MgCl_2$, 5; $NaH_2PO_4$, 1.25; $CaCl_2$, 1.0; glucose, 10; pH 7.2–7.4), transverse NS sections were cut (350 μm) and placed in oxygenated (95% $O_2$–5% $CO_2$) ACSF (in mM: $CaCl_2$, 2; $MgCl_2$, 2; lactate, 4; 25–27 °C) for at least 1 h, and then transferred to a perfusion chamber attached to the stage of a fixed-stage upright microscope (Zeiss Axioskop, Thornwood, NY, USA) where the slice was submerged in continuously flowing oxygenated ACSF (25 °C, 4 mL/min, lactate removed). Cells were visualized with a 40 × water immersion lens, illuminated with near infra-red light (790 nm, Ealing Optics, Hollston, MA, USA) and the image detected with an infra-red-sensitive CCD camera (Hamamatsu C-2400, Bridgewater, NJ, USA). Digital images were stored for subsequent analysis. Cells were typically visualized from 30 to 100 μm below the surface of the slice.

After equilibration for 10 min, a baseline image of the cells was obtained and stored. The slice was then exposed to a different solution depending upon the purpose of the specific experiment. Images were obtained and stored at 5 min intervals for the duration of each experiment (15 min exposure to experimental treatment). In order to quantify changes in response to EAA agonists and the modulatory actions of DA and its receptor agonists, cross-sectional somatic area was measured at each 5 min time point, before and during experimental treatments. Each measurement was made three times on each image and the average value recorded for each cell at each time point. For all quantitative comparisons between experimental and control groups, data were analysed at the 15 min time point during exposure to the experimental treatment. For each experimental group, data were obtained from several animals. Typically, three to seven cells were visualized within each slice and data from several slices from each animal were used in the different experimental groups. Data from controls (NMDA or KA alone) at different concentrations were collected over the course of the experiments to minimize potential influences of systematic differences in data collection procedures. Cells were chosen for measurement before slices were exposed to experimental treatments to minimize sampling bias. As a reliability check in selected cases, cross-sectional area was measured by more than one experimenter. Average areas measured by multiple observers were similar (< 10% difference).

Cross-sectional areas at each time point were converted to percentage change with respect to the baseline area for each cell. There were no consistent differences due to variation between animals and slices within animals. Therefore, for statistical analyses, data were pooled for all cells in experimental and control groups. Differences between means for experimental groups were subsequently analysed with the appropriate analyses of variance (two-way with one repeated measure for NMDA time × concentration analysis and one-way for independent measures for the effects of DA, $D_1$ and $D_2$ agonists) followed by multiple comparisons using the Newman–Keuls method or *t*-tests when only two groups were compared. In the text and tables, values are presented as mean ± standard errors (SE). Differences between means for experimental and control conditions were considered statistically significant when $P < 0.05$. We have published previously extensive analyses of swelling induced by exposure to glutamate receptor agonists (Colwell & Levine, 1996; Colwell *et al*., 1996). The results of the present experiments concerning the properties of swelling induced by NMDA were similar to our published values but based on a completely different data set.

EAA agonists were obtained from Sigma (St Louis, MO, USA) while EAA antagonists were obtained from Tocris (Ballwin, MD, USA). DA, its receptor agonists and antagonists were obtained from RBI (Natick, MA, USA). Concentrations of NMDA (25, 50 and 100 μM) or KA (10 and 25 μM) were based on our previously published studies (Colwell & Levine, 1996; Colwell *et al*., 1996). Two concentrations of DA (30 and 50 μM) and the $D_1$ agonist (SKF 38393; 20 and 30 μM) and three concentrations of the $D_2$ agonist (quinpirole; 10, 20 and 30 μM) were used. These concentrations were chosen based on preliminary experiments and results of the electrophysiological experiments (Cepeda *et al*., 1993, 1998). Concentrations of DA receptor antagonists were 10 μM for SCH 23390, the $D_1$ antagonist and 10 μM for sulpiride, the $D_2$ antagonist. Drugs were freshly prepared, dissolved in standard ACSF and applied in the bath. Na-metabisulphite (50 μM) was added to solutions of DA and SKF 38393 to prevent oxidation.

## Results

Quantitative data were obtained from 1178 NS cells with medium-sized somata (mean maximum diameter < 15 μm; mean cross-sectional area = 99 ± 5 $\mu m^2$; range = 50–130 $\mu m^2$) (Fig. 1, inset). Dendritic spines could not be resolved using IR-DIC optics; however, proximal dendrites were seen in some cells. If these cells are filled with biocytin in a patch pipette, neurons display dendritic fields characteristic of medium-sized spiny neurons (Cepeda *et al*., 1995, 1998). Cells were selected for analysis based on a number of criteria; they had a three-dimensional appearance, somatic borders were clearly delineated, and the nucleus and nucleolus were not visible (Fig. 2). Large neurons (somatic area > 150 $\mu m^2$), were easily distinguished but were not included for quantitative analysis in the present study.

Exposure to ACSF alone for up to 30 min did not change cell area (– 0.3 ± 1.4% change in cross-sectional area, $n = 28$). Bath application of NMDA produced concentration- and time-dependent statistically significant increases in somatic cross-sectional area (Figs 1 and 2A,B, top panels). The swelling at 50 μM exposure to NMDA

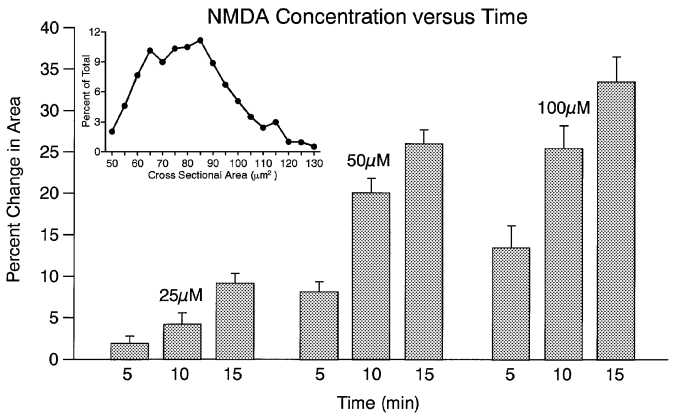


FIG. 1. Time- and concentration-dependent increases in NMDA-induced cell swelling. Inset shows the distribution of cross-sectional areas (in $\mu m^2$) for the 1178 cells examined in all swelling experiments before experimental treatment. Each set of three bars shows the percentage increase in cell cross-sectional area 5, 10 and 15 min after exposure to 25 ($n = 128$), 50 ($n = 158$) or 100 μM ($n = 55$) NMDA. The differences among mean values for concentration, time and the interaction were all statistically significant (two-way analysis of variance; $F_{concentration} = 71$, d.f. = 2/338, $P < 0.0001$; $F_{time} = 39$, d.f. = 2/676, $P < 0.0001$; $F_{concentration \times time} = 4.63$, d.f. = 4/676, $P = 0.0011$).

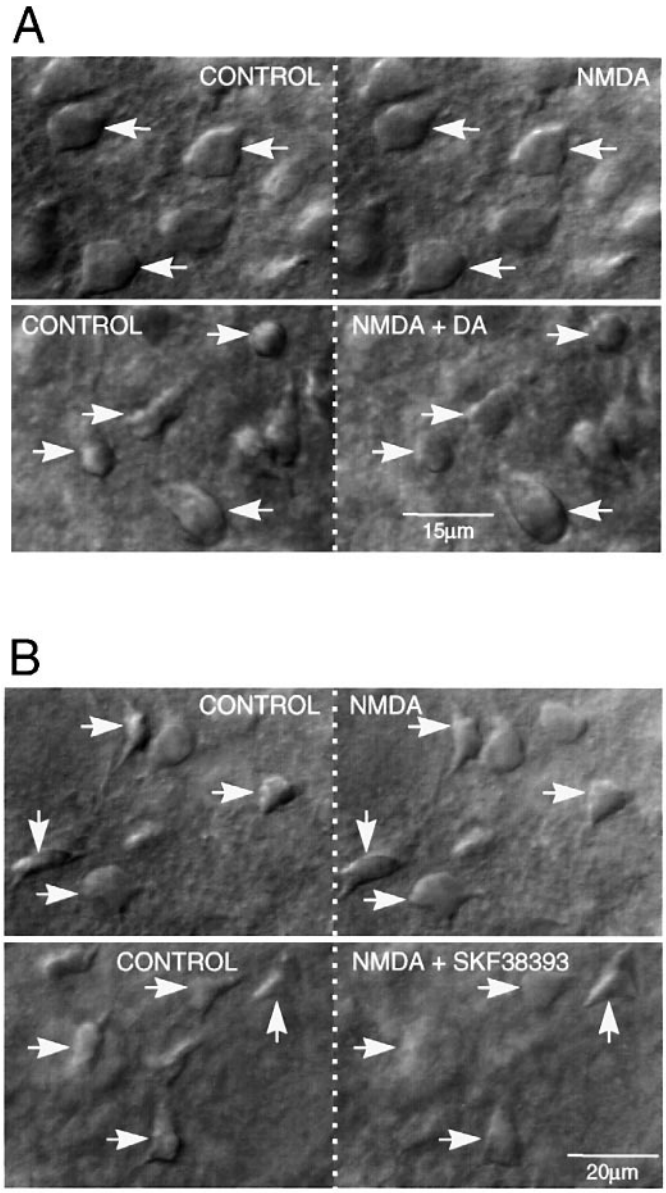


FIG. 2. Examples of enhancement of NMDA-induced cell swelling in the presence of DA or SKF 38393. Arrows in the left and right panels of A and B show the same cells before and after 15 min exposure to treatments. (A) Top two panels illustrate the effect of bath application of NMDA (25 μM) alone. There were slight increases in cross-sectional area. Bottom panels show that cell swelling was enhanced after 15 min exposure to 25 μM NMDA and DA (50 μM). Calibration in the bottom right panel refers to all panels in A. (B) Top two panels illustrate the effect of bath application of NMDA (25 μM). Again, there were slight increases in cross-sectional area. Bottom panels show that cell swelling was enhanced after 15 min exposure to 25 μM NMDA and SKF 38393 (30 μM). Calibration in the bottom right panel refers to all panels in B.

was blocked by the NMDA receptor antagonist, 2-amino-phosphonovalerate (AP5) [26 ± 2% ($n = 158$) for 50 μM NMDA vs. − 1 ± 2% ($n = 26$) for 50 μM NMDA + 20 μM AP5; $t = 7.00$, d.f. = 159, $P < 0.0001$] and significantly enhanced in $Mg^{2+}$-free ACSF [26 ± 2% ($n = 158$) in 50 μM NMDA in standard ACSF vs. 43 ± 4% ($n = 20$) for 50 μM NMDA in $Mg^{2+}$-free ACSF; $t = 2.59$, d.f. = 143, $P = 0.01$] providing evidence that NMDA receptors were involved specifically in the phenomena. In previous studies we demonstrated that NMDA-induced swelling was not affected by 6-cyano-7-nitroquinoxaline-2,3-dione, a non-NMDA receptor antagonist or the metabotropic glutamate receptor antagonist, α-methyl-4-carboxyphenylglycine (Colwell & Levine, 1996; Colwell *et al*., 1996).

Exposure to DA and SKF 38393 enhanced (Fig. 2A,B) while exposure to quinpirole decreased NMDA-induced swelling. In order to minimize ceiling and floor effects, DA, $D_1$ and $D_2$ modulation of NMDA-induced swelling were tested at different concentrations of NMDA. Pilot experiments indicated that DA enhancement of NMDA-induced swelling with $D_2$ agonists was minimal when cells were exposed to 100 μM of NMDA and attenuation of swelling was not marked when cells were exposed to 25 μM NMDA. Therefore, effects of DA and SKF 38393 were examined at 25 and 50 μM NMDA and effects of quinpirole examined only at 50 μM NMDA.

Alone, DA did not produce a significant change in average cross-sectional area but when DA was combined with 25 μM NMDA, swelling increased significantly (Figs 2A and 3A). This effect occurred at a concentration of 50 μM DA but not at 30 μM DA. The effect could be blocked by pre-exposure to the $D_1$ receptor antagonist SCH 23390 (10 μM), suggesting it was mediated by activation of $D_1$ receptors (Fig. 3A). DA (50 μM) had almost no effect on swelling induced by 50 μM NMDA [26 ± 2% ($n = 158$) for 50 μM NMDA vs. 27 ± 4% ($n = 21$) for 50 μM NMDA + 50 μM DA]. This could be explained by ceiling effects (i.e. little additional swelling could occur) or by the ability of DA to activate multiple receptor subtypes as SKF 38393 invariably enhanced swelling (see below).

The enhancement of NMDA-induced swelling by DA was mimicked by SKF 38393 (20–30 μM) (Figs 2B and 3B). At both 25 and 50 μM NMDA, SKF 38393 enhanced the swelling. Alone, SKF 38393 did not alter significantly cross-sectional area (Fig. 3B). The enhancement of NMDA-induced swelling by SKF 38393 also was blocked by pre-exposure to SCH 23390 (10 μM) (Fig. 3B). In contrast, the $D_2$ receptor agonist, quinpirole produced a dose-dependent decrease in NMDA-induced cell swelling (Fig. 3C). Again, quinpirole alone did not alter cross-sectional area. The effects of quinpirole could be partially antagonized by pretreatment with sulpiride, a $D_2$ receptor antagonist (Fig. 3C).

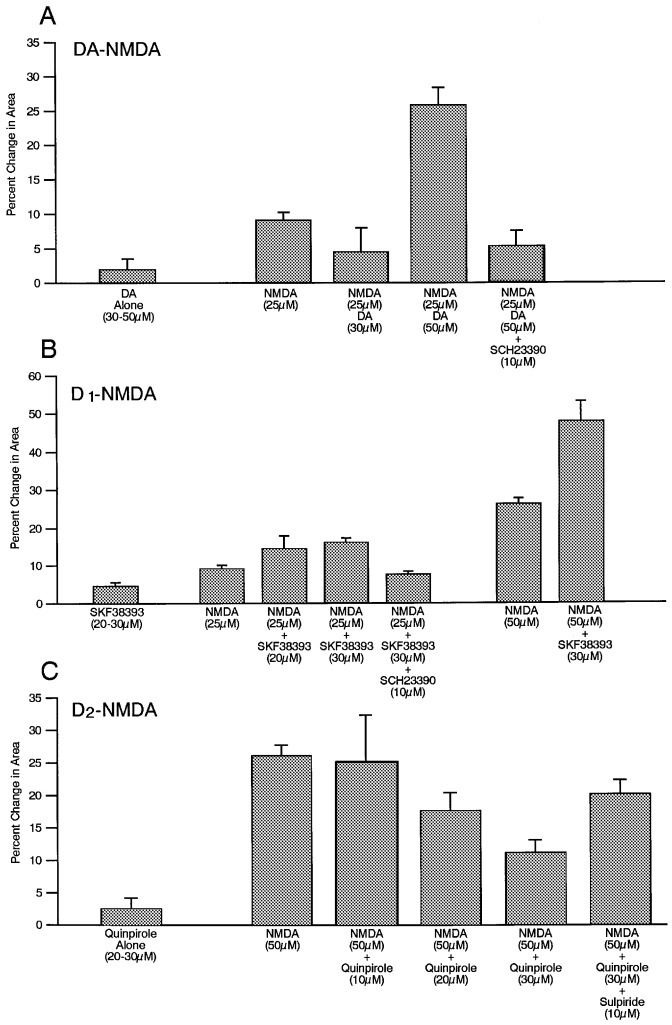


Fig. 3. Effects of DA, SKF 38393 and quinpirole on NMDA-induced cell swelling. (A) Alone DA ($n = 67$) had no statistically significant effect on cell area. When NMDA (25 μM) was combined with 30 μM DA ($n = 11$), a small variable decrease in NMDA-induced cell swelling occurred. This effect was not statistically significant. When NMDA (25 μM) was combined with 50 μM DA ($n = 51$), the increase in cell swelling was statistically significant (one-way analysis of variance, $F = 22$, d.f. = 4/262, $P < 0.0001$; Newman–Keuls multiple comparison, $P < 0.05$). The enhancement of NMDA-induced cell swelling by 50 μM DA was blocked by pretreatment with the $D_1$ agonist SCH 23390 (10 μM) ($n = 35$). (B) Exposure to SKF 38393 ($n = 38$) alone had no statistically significant effect on cell area. When NMDA (25 μM) was combined with either 20 ($n = 28$) or 30 μM SKF 38393 ($n = 95$) there were statistically significant increases in NMDA-induced cell swelling [one way analysis of variance; $F = 7.31$, d.f. = 4/326, $P < 0.001$; Newman–Keuls pairwise comparisons, $P < 0.05$ for NMDA (25 μM) vs. NMDA (25 μM) + SKF 38393 (20 μM) and $P < 0.05$ for NMDA (25 μM) vs. NMDA (25 μM) + SKF 38393 (30 μM)]. The effect of 30 μM SKF 38393 was blocked by pretreatment with SCH 23390 (10 μM) ($n = 59$). When NMDA (50 μM) was combined with 30 μM SKF 38393 ($n = 25$) there was also a statistically significant increase in NMDA-induced cell swelling ($t = -4.07$, d.f. = 149, $P < 0.0001$). Note that the ordinate for B is plotted on a different scale than ordinates for A and C. (C) Exposure to quinpirole alone ($n = 16$) produced no statistically significant effect on cell area. When combined with 50 μM NMDA, quinpirole produced a concentration-dependent statistically significant decrease in cell swelling [one-way analysis of variance; $F = 9.45$, d.f. = 5/254, $P < 0.0001$; Newman–Keuls pairwise comparisons indicated quinpirole-induced decreases in area were statistically significant for 20 ($n = 37$) ($P < 0.05$) and 30 μM ($n = 41$) ($P < 0.05$) compared with 50 μM NMDA alone]. The effect of quinpirole was partially blocked when the slice was pretreated with sulpiride (10 μM) ($n = 30$), a $D_2$ antagonist [$P < 0.05$ for comparison between NMDA (50 μM) + quinpirole (30 μM) vs. NMDA (50 μM) + quinpirole (30 μM) + sulpiride (10 μM)].

Since we had previously shown that DA receptor activation may affect differentially responses evoked by NMDA and non-NMDA receptor agonists, in additional experiments we determined whether or not the enhancement of cell swelling was specific for NMDA receptor activation. As observed with NMDA, bath application of KA (10 and 25 μM) also induced cell swelling. Swelling induced by 25 μM was significantly greater than swelling induced by 10 μM KA [9 ± 2% ($n = 26$) vs. 28 ± 3% ($n = 33$) for 10 and 25 μM, respectively; $t = 5.70$, d.f. = 57, $P < 0.001$] When KA (25 μM) was applied in conjunction with DA (50 μM) cell swelling was slightly decreased but the effect was not statistically significant [28 ± 3% ($n = 33$) vs. 22 ± 3% ($n = 30$) for 25 μM KA without and with DA, respectively]. We also assessed the effects of SKF 38393 on KA-induced swelling. At the low concentration of KA (10 μM), SKF 38393 (20 μM) significantly enhanced swelling [9 ± 2% ($n = 26$) vs. 17 ± 2% ($n = 23$) for 10 μM KA without and with SKF 38393, respectively; $t = 3.52$, d.f. = 47, $P < 0.001$]. At the higher concentration of KA, SKF 38393 produced no effect [28 ± 3% ($n = 33$) vs. 29 ± 2% ($n = 41$) for 25 μM KA without and with SKF 38393, respectively]. Thus, there may be a ceiling effect on the actions of SKF 38393 in enhancing cell swelling.

Activation of $D_2$ receptors with quinpirole produced a concentration-dependent decrease in KA-induced cell swelling. Application of KA alone (25 μM) caused 36 ± 5% swelling ($n = 21$). Simultaneous treatment with increasing concentrations of quinpirole produced a statistically significant decrease in KA-induced swelling (27 ± 3%, 16 ± 2% and 7.1 ± 2.5% at 10, 20 and 30 μM quinpirole, respectively; $F = 13.4$, d.f. = 3/61, $P < 0.001$). The subsequent Newman–Keuls test indicated that only the differences between KA alone vs. KA + quinpirole at 20 and 30 μM and KA + quinpirole at 10 μM vs. KA + quinpirole at 30 μM were statistically significant.

A potential problem with the present results is that the outcomes are based on data obtained from animals between postnatal days 12 and 18. The need to use very young animals is a limitation of infrared videomicroscopy. In the rat NS, myelination increases considerably after the third postnatal week and thus, it is easier to visualize cells in younger tissue. This problem means that it may be difficult to generalize the findings of these experiments to adult animals. To address this problem we performed a series of experiments in rats 5–8 weeks of age. We examined the ability of SKF 38393 to enhance NMDA-induced cell swelling and the ability of quinpirole to reduce KA-induced swelling. As expected, it was more difficult to visualize cells and more slices had to be used to obtain the data than would have been necessary in younger tissue. In older animals, NMDA (25 μM) induced an average increase of 23 ± 2.4% ($n = 23$). In the presence of SKF 38393 (30 μM), swelling was significantly increased (35 ± 2.7%, $n = 23$; $t = 11.3$, d.f. = 44, $P = 0.003$ vs. NMDA alone). KA (25 μM) induced an average increase of 25 ± 3.0% ($n = 24$) when applied alone. In the presence of quinpirole (30 μM), KA-induced swelling was significantly reduced (10 ± 3.1%, $n = 24$; $t = 3.49$, d.f. = 45, $P = 0.001$ vs. KA alone). Thus, the differential effects of activation of $D_1$ or $D_2$ receptors on NMDA and KA receptor-mediated cell swelling are not idiosyncratic to the use of young tissue but occur in older animals as well.

One other methodological issue that could have affected DA modulation of cell swelling was the temperature at which experiments were conducted. Experiments were performed at room temperature (25–27 °C) because we were interested in comparing the results of cell swelling with parallel electrophysiological recordings (Cepeda *et al.*, 1998) using patch-clamp techniques which produce better seals at the lower temperatures. As a check that our findings were not simply due to the lower temperature in a group of additional older

rats (6–8 weeks) we examined the ability of SKF 38393 and quinpirole to alter cell swelling produced by application of NMDA and KA, respectively, at temperatures of 36–38 °C. NMDA (25 μM) induced an average increase of 26 ± 2.1% ($n$ = 19). In the presence of SKF 38393 (30 μM), swelling was significantly increased (34 ± 3.3%, $n$ = 19; $t$ = 2.04, d.f. = 36, $P$ = 0.049 vs. NMDA alone). KA (25 μM) induced an average increase of 43 ± 3.1% ($n$ = 9) when applied alone. In the presence of quinpirole (30 μM), KA-induced swelling was significantly reduced (25 ± 4.6%, $n$ = 8; t = 3.28, d.f. = 15, $P$ = 0.005 vs. KA alone). Thus, the differential effects of activation of $D_1$ or $D_2$ receptors on NMDA and KA receptor-mediated cell swelling occur at higher temperatures.

## Discussion

The major outcome of these experiments was that EAA-induced cell swelling is modulated by DA and its selective receptor agonists. DA and SKF 38393 enhanced NMDA-induced swelling. This effect was blocked by the $D_1$ antagonist. In contrast, activation of $D_2$ receptors produced a consistent decrease in NMDA-induced cell swelling that could be partially blocked by a $D_2$ antagonist. SKF38393 increased KA-induced swelling only at exposures to a low concentration of KA (10 μM). DA slightly reduced KA-induced cell swelling. In contrast, the $D_2$ agonist reduced KA-induced swelling in a concentration-dependent manner.

These findings are generally in agreement with our previous electrophysiological studies showing that the direction of the modulatory actions of DA are dependent upon the EAA and DA receptor subtypes activated (Cepeda *et al*., 1993, 1998; Levine *et al*., 1996b). We have hypothesized that the combination of subtypes of EAA and DA receptors activated determines the direction of DA modulation. We have shown that DA potentiates responses mediated by activation of NMDA receptors, but attenuates responses mediated by activation of non-NMDA receptors (Cepeda *et al*., 1993; Levine *et al*., 1996a,b). The effects of DA on responses mediated by NMDA receptor activation are mimicked by application of $D_1$ receptor agonists and are blocked by a $D_1$ antagonist. The ability of DA and $D_1$ agonists to potentiate responses mediated by activation of NMDA receptors also is reduced significantly in mutant mice lacking $D_{1A}$ DA receptors (Levine *et al*., 1996a). More recently we confirmed the effects of DA on NMDA-induced currents using whole-cell voltage clamp recordings and infra-red videomicroscopy (Cepeda *et al*., 1998).

The use of tissue from developing animals influences the interpretation of the findings as it could be argued that EAA and DA receptor function has not reached adult levels by postnatal days 12–18. To address this question we examined DA modulation of swelling in 5–8-week-old rats. $D_1$-mediated enhancement of NMDA-induced cell swelling and $D_2$-attenuation of KA-induced swelling were present in the older tissue. In addition, a number of other lines of evidence indicate that the present findings can be extrapolated to the adult. Previous work indicates that by postnatal day 14 many of the functional properties of NS neurons have developed (Walsh *et al*., 1989; Tepper & Trent, 1993). Of more importance, in recent experiments we found that NMDA- and KA-induced swelling and membrane currents develop primarily over the first 14 postnatal days in the rat NS (Cepeda *et al*., 1996; Levine *et al*., 1996c; Colwell *et al*., 1998). The ability of DA and its receptor agonists to modulate these responses appears to develop in parallel (unpublished observations). Although earlier studies have shown that young NS tissue is more sensitive to NMDA damage than older tissue (McDonald *et al*., 1992), these experiments were based on *in vivo* infusions and age-related differences in diffusion were not controlled. Furthermore, the general agreement between the present findings and our electrophysiological data obtained from adult rats (Cepeda *et al*., 1993; Levine *et al*., 1996a,b) provides additional validation that developmental variables may not have markedly influenced our conclusions.

As pointed out above there is considerable evidence to support a potential role for DA in modulating or inducing neurotoxicity (Gibb *et al*., 1990; Olney, 1990; Ryan *et al*., 1990; Marshall *et al*., 1993; Ohmori *et al*., 1993). The present studies support a modulatory role for DA as alone it did not cause cell swelling. Furthermore, similar to the present study the evidence indicates that activation of $D_1$ receptors enhances toxicity or may induce neuropathological changes (Kelley *et al*., 1990). Activation of $D_2$ receptors had little effect on neurotoxicity (Witkin *et al*., 1993), a result that was different from the findings of the present study in which activation of $D_2$ receptors attenuated cell swelling.

In the present study, IR DIC videomicroscopy was used to examine cell swelling in a brain slice preparation as a rapid measure of toxicity. This swelling is believed to be an early step in a complex cascade of events which can eventually lead to excitotoxic cell death (Choi, 1988; Rothman, 1992). We did not examine directly the role of DA in the final outcome of toxicity as cells were visualized for very short periods of time after exposure to NMDA and DA agonists and techniques that would reveal dead or dying neurons were not used. Previously we have used Trypan blue to provide further support for the view that NMDA- and KA-induced cell swelling is an early marker of excitotoxic damage (Colwell *et al*., 1996). This dye is normally excluded from healthy cells but is taken up by dead cells and is widely used in cell culture as a marker of cell death (Phillips, 1973). Cells which exhibited clear borders and phase brightness never took up the dye. In contrast, cells exhibiting indistinct borders and low phase brightness with the nucleus visible became stained when exposed to Trypan blue and were likely to have been damaged or dead. Exposure to NMDA dramatically increased the number of cells which took up the Trypan blue. In some cases, we found that a cell which excluded Trypan blue initially, took up the dye after exposure to NMDA. However, some cells which showed NMDA-induced swelling still retained the ability to exclude Trypan blue. Thus, the events appear to be closely associated and are consistent with the hypothesis that extensive swelling is an initial step in a cascade of events which can, but does not always, lead to cell death.

The great majority of NS neurons are medium spiny projection neurons (Gerfen, 1992) and it is likely that most of the data in the present study were collected from this cell type. When biocytin or Lucifer yellow was injected through a patch pipette labelled cells were medium-sized neurons (Cepeda *et al*., 1995, 1998). Specific classes of these medium-sized cells have been shown to be susceptible to excitotoxic damage and are those at risk for degeneration in Huntington's disease (DiFiglia, 1990). Large interneurons were systematically excluded from the present data set. These cells seem to be spared of degenerative changes in Huntington's disease and from other studies appear less susceptible to excitotoxic damage than the medium-sized cells (Graveland *et al*., 1985). In support of these observations, in preliminary experiments we found that NMDA does not produce cell swelling in large neurons. This observation also provides further support to the cell swelling assay as a valid method to study excitotoxicity.

There are different ways that DA could contribute to excitotoxicity in the NS. The simplest explanation is that DA and $D_1$ agonists enhance NMDA-induced currents which begin the sequence of events that leads to the osmotic changes that ultimately cause the cell swelling. Although previous work has emphasized the inhibitory role of DA in the NS (Calabresi *et al*., 1987), other findings provided

evidence that DA has multiple actions (Chiodo & Berger, 1986; Akaike *et al*., 1987). Our laboratory was the first to demonstrate that the modulatory effects of DA depend on which type of glutamate or DA receptor is activated. In that sense, DA could produce excitatory and inhibitory effects on the same neuron. Recent work also indicates that DA and $D_1$ receptor agonists have potentiating effects when the cell membrane is depolarized (Hernández-Lopez *et al*., 1997). There is strong evidence that some of these effects are due to the ability of DA to enhance an 'L' type of voltage-dependent $Ca^{2+}$ current (Surmeier *et al*., 1995; Cepeda *et al*., 1998). Thus, sustained activation of NMDA and DA receptors ($D_1$ subtype) may be deleterious for the cell. Simultaneous activation of these receptor subtypes can induce potentially lethal levels of $Ca^{2+}$ influx.

An alternative but also attractive hypothesis for excitotoxic damage involves energy failure (Beal *et al*., 1993). According to this hypothesis, impairment of neuronal energy metabolism results in reduced levels of ATP. A consequence of interfering with the function of $Na^+/K^+$ ATPase is a defective repolarization which could lead to prolonged activation of $Ca^{2+}$ channels and enhanced influx through NMDA channels. One effect of DA is inhibition of $Na^+/K^+$ ATPase activity in isolated NS neurons (Bertorello *et al*., 1990) which would predispose toward enhancement of excitotoxicity. Furthermore, there is recent evidence that drugs that interfere with this ionic pump enhance the membrane depolarizations and inward currents induced by subcritical concentrations of EAAs (Calabresi *et al*., 1995). Finally, DA is a potent reversible inhibitor of mitochondrial respiration (Ben-Shachar *et al*., 1995). Thus, some of the effects of DA receptor activation may be due to alterations in ionic pumps.

Excitotoxic degeneration in the NS has received considerable attention because of its possible association with the major pathological sequelae of Huntington's disease (DiFiglia, 1990; Freese *et al*., 1990; Koroshetz *et al*., 1990; Beal *et al*., 1993). The present experiments demonstrate that DA directly modulates EAA-induced excitotoxicity. There are potential associations among the present findings and recent work on Huntington's disease. There is now evidence that huntingtin expression in a striatal-neuroblastoma hybrid cell line is altered after treatment with forskolin (Kim *et al*., 1997). We have shown previously, that forskolin enhances responses to glutamate receptor agonists (Colwell & Levine, 1995). Forskolin enhances cyclic adenosine monophosphate, activating protein kinase A, the same transduction system increased after activation of $D_1$ DA receptors (Stoof & Kebabian, 1981). Thus, combined activation of specific glutamate and DA receptors could predispose neurons to excitotoxic damage and cause abnormal expression of huntingtin. The present findings also have potential importance in Parkinson's disease as drugs that alter glutamate neurotransmission have been used to improve classical DA therapy in this disorder (Schneider & Roeltgen, 1993; Blandini *et al*., 1996).

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## Abbreviations

| | |
|---|---|
| ACSF | artificial cerebrospinal fluid |
| AP5 | 2-amino-phosphonovalerate |
| DA | dopamine |
| EAA | excitatory amino acid |
| IR-DIC | infra-red-differential interference contrast |
| KA | kainate |
| NMDA | *N*-methyl-D-aspartate |
| NS | neostriatum |
| SE | standard error of the mean |

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