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Letter to Neuroscience

CHRONIC INTRASTRIATAL QUINOLINIC ACID PRODUCES REVERSIBLE CHANGES IN PERIKARYAL CALBINDIN AND PARVALBUMIN IMMUNOREACTIVITY

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We recently reported the use of a chronic dialytic delivery system for intrastriatal administration of quinolinic acid in the rat.[3,4] This system produces neurodegeneration with some characteristics similar to *post mortem* brain tissue from Huntington's disease patients, including reduced cytochrome oxidase staining, a decreased number of Nissl-stained neurons, and relative sparing of striatal NADPH-diaphorase containing neurons.[4] The present findings show that chronic dialytic delivery of quinolinic acid also produces a Huntington's disease-like pattern of reduced calbindin and parvalbumin perikaryal immunoreactivity that is reversed in rats allowed four to eight weeks' recovery after cessation of quinolinic acid. Furthermore, cytochrome oxidase staining and the number of Nissl-stained cells were unchanged in the region of transient calbindin and parvalbumin immunoreactive perikaryal staining alterations. These results suggest that changes in calbindin and parvalbumin perikaryal immunoreactivity provide a relatively sensitive measure of quinolinic acid induced neurotoxicity. The reversible nature of reduced perikaryal immunoreactivity suggests a premorbid state of neurotoxicity, possibly marked by cellular redistribution of calbindin and parvalbumin.

Quinolinic acid acts as an agonist at *N*-methyl-D-aspartate (NMDA) receptors, causing an increase in intracellular calcium concentration.[20] To maintain intracellular ionic equilibrium, calcium can be sequestered by intracellular organelles or buffered by calcium binding proteins.[1,2] Excessive calcium influx through the NMDA receptor complex results in excitotoxicity.[10,15,21,22] Cells containing the calcium buffering protein, calbindin, are reduced in *post mortem* striatal tissue from all grades of Huntington's disease (HD).[13,19] Cells containing parvalbumin, another calcium buffering protein, appear more resistant and are reduced only in tissue from advanced HD.[9]

In a previous report, Nissl staining showed that chronic intrastriatal dialytic delivery of 4 mM quinolinic acid had no effect on neuronal density, while 15 mM quinolinic acid produced a decrease in neuronal density that was restricted to an area approximately 400 μm radial to the dialysis probe in the rat.[4] In the present experiments, quantitation of Nissl-stained neuron density revealed findings identical to the previous study for both 4 and 15 mM concentrations of quinolinic acid. However, as shown in Fig. 1, perikaryal calbindin and parvalbumin immunoreactivity was significantly reduced throughout a region 400 μm radial to the dialysis probe in animals receiving 4 mM quinolinic acid (calbindin: control 170 ± 9.55 vs quinolinic acid 73.3 ± 11.19, $P < 0.001$; parvalbumin: control 24.25 ± 2.33 vs quinolinic acid 14.3 ± 2.68, $P < 0.005$; mean number of perikarya ± S.E.M., two-tailed Student's paired t-test). Animals that received 15 mM quinolinic acid showed reduced calbindin and parvalbumin perikaryal immunoreactivity throughout most of the striatum (Table 1, Fig. 2).

There was a dose-dependent effect of quinolinic acid on cytochrome oxidase staining. Analysis of brain selections containing the dialysis probe tract revealed that 15 mM quinolinic acid produced a significantly greater area of decreased staining than did 4 mM quinolinic acid (Veh 12.8 ± 2.53 mm² vs 4 mM 27.1 ± 1.22 mm², mean ± S.E.M., $P < 0.001$; 4 mM 27.1 ± 1.22 mm² vs 15 mM 56.1 ± 5.91 mm², $P < 0.001$; Student's unpaired t-test). For both doses, the decrease in perikaryal calbindin and parvalbumin immunoreactivity was apparent well beyond the area of decreased cytochrome oxidase staining.

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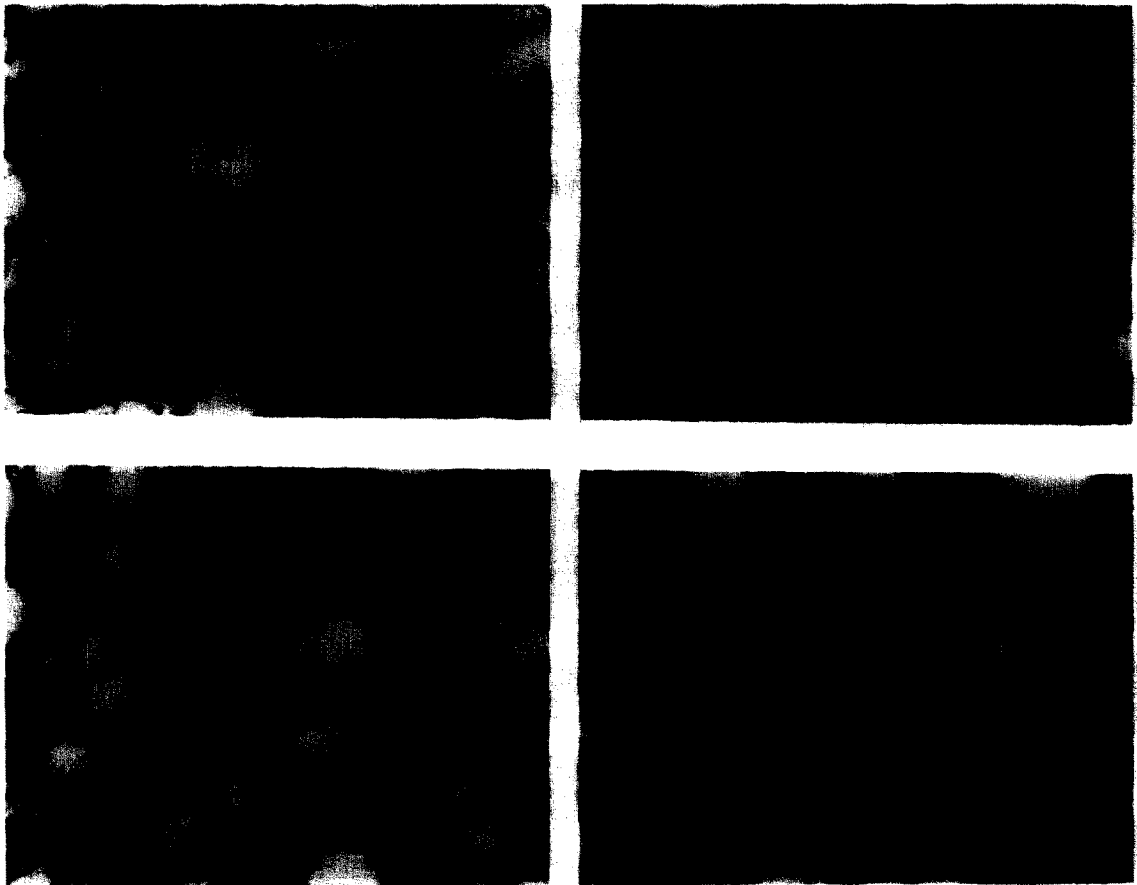*Abbreviations*: HD, Huntington's disease; NMDA, *N*-methyl-D-aspartate.

Fig. 1. Data from Experiment 1 showed that 21 days of 4 mM quinolinic acid produced a significant decrease in calbindin and parvalbumin immunoreactive perikarya compared to control striata. Detailed methods for the dialytic delivery system are described elsewhere.[3] Perikarya were analysed in a 1.02×10^{-1} mm³ volume of striatal tissue 400 μm radial from the necrotic core or a homologous region in the contralateral (control) striatum of 12 Sprague–Dawley rats (Harlan Sprague–Dawley, Inc. Indianapolis, IN). In the control striatum calbindin (A) and parvalbumin (C) immunoreactive staining is normal. Exposure to 4 mM quinolinic acid for 21 days produced a significant decrease in calbindin (B) and parvalbumin (D) immunoreactive perikarya and an increase in neuropil staining. Calbindin and parvalbumin antibodies (Sigma) were diluted 1:2000 and immunohistochemical assays performed using Vectastain mouse IgG Elite ABC kit (Vector Labs, Inc.) according to manufacturer's instructions. There was no significant difference in the number of nissl-stained cells (not shown) in control (186.5 ± 10.46) vs quinolinic acid-exposed (160.3 ± 14.82) striata. Scale bar = 100 μm.

Table 1. Combined data from Experiment 2 showing a relatively rapid and sustained loss, and Experiment 3 showing the subsequent recovery, of calbindin and parvalbumin immunoreactive perikaryal staining in response to 15 mM quinolinic acid

	Calbindin		Parvalbumin	
	Quinolinic acid	Control	Quinolinic acid	Control
Time course				
5 days ($n = 6$)	84.0 ± 16.8**	246.0 ± 15.4	9.2 ± 2.4**	25.8 ± 2.7
10 days ($n = 4$)	48.0 ± 24.4*	212.1 ± 26.1	14.4 ± 5.5	25.0 ± 2.5
15 days ($n = 4$)	73.8 ± 19.1*	251.0 ± 44.4	15.7 ± 0.9*	29.3 ± 2.4
21 days ($n = 7$)	65.4 ± 23.3**	246.6 ± 24.6	11.5 ± 2.0*	19.0 ± 0.8
Recovered				
4 weeks ($n = 5$)	162.4 ± 20.1	209.6 ± 30.5	15.8 ± 1.2	16.2 ± 1.8
8 weeks ($n = 4$)	118.3 ± 33.9	193.3 ± 36.3	18.0 ± 1.8	19.8 ± 3.3

Perikarya were analysed in a 1.02×10^{-1} mm³ volume of tissue from the ventral lateral striatum approximately 1 mm caudal from the necrotic core. Two-tailed paired Student's t-tests were used to determine significant changes in the number of calbindin and parvalbumin immunoreactive perikarya between control and quinolinic acid exposed striata (*$P < 0.05$ **$P < 0.005$).

Cresyl Violet stained sections were also analysed in animals receiving 21 days ($n = 7$) administration. There was no significant difference in the number of Nissl-stained cells in control (110.4 ± 4.58) vs quinolinic acid exposed (98.3 ± 10.45) striata.
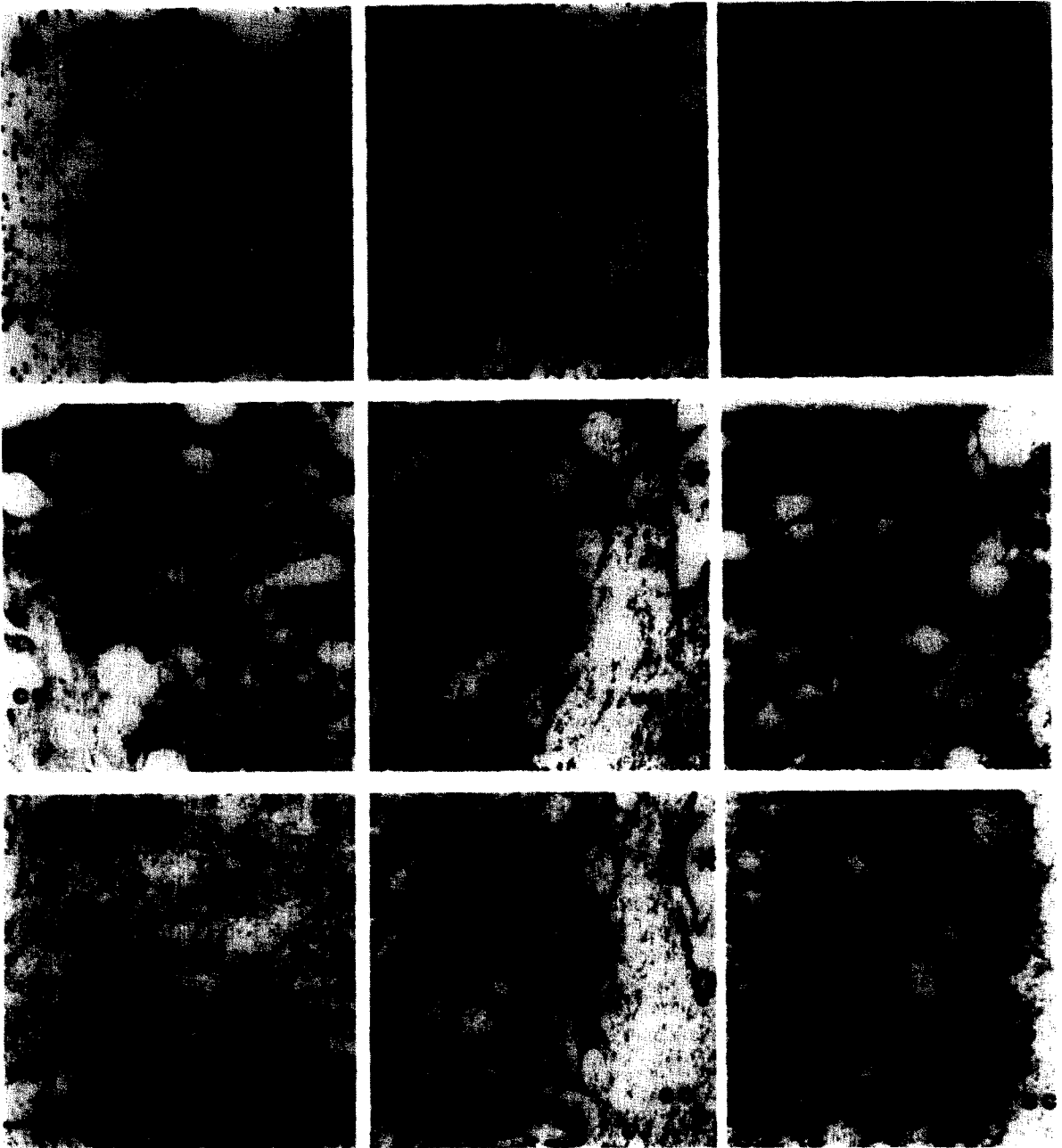
Fig. 2. Representative sections compare posterior lateral striatal tissue after: 21 days vehicle, 21 days 15 mM quinolinic acid, and 21 days 15 mM quinolinic acid plus four weeks recovery. Cresyl Violet staining was similar across the three groups. Calbindin and parvalbumin immunoreactive perikarya decreased after 21 days 21 mM quinolinic acid, but not different after 15 mM quinolinic acid plus four weeks' recovery, when compared to vehicle. Cresyl Violet staining after 21 days vehicle (A), 21 days quinolinic acid (B), 21 days quinolinic acid + four weeks of recovery (C). Calbindin immunoreactive staining 21 days vehicle (D), 21 days quinolinic acid (E), 21 days quinolinic acid + four weeks recovery (F). Parvalbumin immunoreactive staining 21 days vehicle (G), 21 days quinolinic acid (H) 21 days quinolinic acid + four weeks recovery (I). cc, Corpus callosum. *Blood vessel provides a landmark for comparison of consecutive 40-μm sections from a brain exposed to 21 days quinolinic acid. Scale bar = 100 μm.

A time course evaluation of animals receiving 15 mM quinolinic acid showed that the decrease in calbindin and parvalbumin perikaryal immunoreactivity occurs relatively rapidly (within five days), and is sustained throughout the 21 day course of quinolinic acid delivery (Table 1).

The ventrolateral region of the caudal striatum showed no change in the density of Nissl-stained cells

but decreased perikaryal calbindin and parvalbumin immunoreactivity after 21 days of 15 mM quinolinic acid compared to vehicle administration. If animals were allowed to survive either four or eight weeks after cessation of quinolinic acid administration, the number of immunoreactive calbindin and parvalbumin perikarya in this brain region was not different from the vehicle-treated striatum (Table 1, Fig. 2). The number of calbindin and parvalbumin immunoreactive perikarya were not different between four- and eight-week animals (Table 1).

The present results show that chronic dialytic delivery of quinolinic acid can decrease the number of calbindin and parvalbumin immunoreactive perikarya. The reversible nature of this effect suggests a change in production or distribution of these intracellular proteins. This hypothesis is further supported by the findings that there is no decrease in cytochrome oxidase staining or in the number of Nissl-stained cells in regions exhibiting transient changes in calcium binding proteins.

Analysis of striata exposed to chronic dialytic delivery of quinolinic acid reveals three primary regions of specific neuronal reactions. The first region, a central necrotic core directly adjacent to the probe tract, is marked by inflammatory infiltrate and a lack of neurons. The second region, previously referred to as the transition zone,[4,5] is marked by an absence of perikaryal staining for parvalbumin and calbindin, a reduction in the number of Nissl-stained cells and a decrease in cytochrome oxidase staining. Though many Nissl-stained neurons in the transition zone appear structurally intact, a decrease in cytochrome oxidase staining suggests metabolic impairment. Beyond the transition zone is the third region, marked only by a decrease in perikaryal calbindin and parvalbumin immunoreactivity. However, unlike the quinolinic acid-induced decrease in cytochrome oxidase and Nissl cell staining in the transition zone, decreased calbindin and parvalbumin staining in the region outside the transition zone is in part reversible.

There was also an increase in calbindin and parvalbumin neuropil staining throughout the striatum with both doses of quinolinic acid, and at all time points after 15 mM quinolinic acid delivery. This increase was also apparent after acute quinolinic acid injections,[23] and may have resulted from immunohistochemical neuropil staining caused by immunoglobulin extravasation.[18]

There are at least two explanations for the transient decrease in perikaryal calbindin and parvalbumin immunoreactivity following quinolinic acid administration. First, it is possible that some cells were excluded from analysis because they were not distinguished from increased neuropil staining. However, it is unlikely that a significant number of cells were excluded since the increase in neuropil staining at no time exceeded the intensity of staining seen in the vast majority of perikarya from the

homologous region of the control striatum; and some immunoreactive neurons could be clearly seen against the increased background on the lesion side (Figs 1, 2).

Alternatively, the transient decrease in perikaryal calbindin and parvalbumin immunoreactivity may be caused by a change in distribution of these proteins within neurons. When stimulated, NMDA receptors allow an influx of calcium into dendritic processes where they are concentrated.[6,22] Dendritic processes, which are capable of independent regulation of changes in intracellular calcium,[8,14] could recruit calcium buffering proteins. Calbindin exhibits characteristics of a fast mobile calcium buffering protein, capable of diffusing from surrounding cytoplasm to local sites of calcium influx.[17] Ferrante and colleagues[7] found the intensity of calbindin immunoreactivity shifts to distal dendritic arbors in HD striatal tissue. A similar calbindin and parvalbumin shift in response to quinolinic acid could explain the observations of decreased perikaryal staining and increased neuropil staining.

It is also interesting to note that two weeks after acute intrastriatal quinolinic acid injection, levels of somatostatin and the glutamic acid decarboxylase isoforms GAD65 and GAD67 were decreased,[16] whereas in a similar paradigm calbindin levels were unchanged.[11] It is possible that exposure to quinolinic acid may produce long-term effects on production of some cellular components, while producing only temporary changes in production or distribution of others.

Calbindin-containing neurons appear less resistant than parvalbumin neurons to the neurodegenerative effects of HD.[9,19] The present results reveal that chronic quinolinic acid administration also reduces a greater percentage of calbindin- compared to parvalbumin-immunoreactive perikarya (Table 1). These results strengthen the contention that the pattern of neuronal vulnerability in HD is shared by striatal tissue exposed to chronic NMDA agonist administration.

The importance of assessing physiological neuronal states that precede cell death associated with neurodegenerative disorders has recently been discussed.[12] In the present report, reversible quinolinic acid induced changes in calbindin and parvalbumin immunoreactivity may represent one such premorbid neuronal state. Further assessment of these changes may offer new insight into the neurodegenerative processes of HD.

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