was 63.8 ± 8.23 and 90.1 ± 10.46 fmol/mg protein for control and ethanol-treated cells respectively; $P < 0.05$, paired $t$-test) without a change in receptor affinity ($K_D$ was 178 ± 16.2 and 200 ± 33.6 pM for control and ethanol-treated cells respectively).

The *in vitro* addition of ethanol inhibits net synaptosomal $^{45}Ca^{2+}$ uptake [2–5]. Of the two phases of synaptosomal calcium uptake, the first phase was more sensitive to ethanol. The *in vitro* addition of 200 mM ethanol significantly inhibited first phase uptake 41%, but only caused a non-significant (17%) reduction in second phase uptake. In addition, 800 mM ethanol abolished first phase uptake, while only causing a 61% inhibition of the second phase. Since the first phase of potassium-stimulated $^{45}Ca^{2+}$ uptake represents uptake through voltage-dependent calcium channels [6] and the second phase represents $Na^+/Ca^{2+}$ exchange [7], the data indicate that the calcium channel is the primary site of action of ethanol on calcium uptake.

The importance of the calcium channel in the actions of ethanol is further indicated by the response of PC12 cells to chronic ethanol administration. In confirmation of a recent report [14], which appeared during preparation of this manuscript, exposure of PC12 cells to ethanol resulted in an increase in net $^{45}Ca^{2+}$ uptake that was due to an increase in the number of voltage-dependent calcium channels per cell as indicated by the increase in the density of binding sites for the calcium channel ligand [$^3$H]PN 200-110. Although Messing *et al.* [14] observed no difference in the potency of *in vitro* ethanol after chronic ethanol treatment, in the present study chronic exposure of PC12 cells to ethanol increased the sensitivity of the voltage-dependent calcium channels to *in vitro* ethanol. A similar increase in sensitivity of adenylate cyclase to *in vitro* ethanol was also observed in PC12 cells chronically exposed to ethanol (R. A. Rabin, manuscript submitted). The increased sensitivity was not due to residual ethanol from the chronic treatment since the inhibitory effects of *in vitro* ethanol on $^{45}Ca^{2+}$ were readily reversible by rinsing the cells (data not shown). The reason for this discrepancy is unclear but may be due to methodological differences or the use of different subclones of the PC12 cells especially since, in contrast to Messing *et al.*, we are unable to maintain viable cells in 200 mM ethanol.

The response of PC12 cells to chronic ethanol appears to be at odds with results obtained using animals. Although an increase in net calcium uptake after chronic ethanol administration was observed in liver microsomes [15], studies with synaptosomes showed either a decrease in net $^{45}Ca^{2+}$ uptake [2] or no change [4]. Results with PC12 cells represent the direct effects of ethanol, whereas ethanol has a wide variety of actions *in vivo* including changes in hormonal balance and systemic metabolism. Thus, the *in vivo* effects of chronic ethnaol exposure on calcium uptake may not be due to a direct neuronal action of ethanol, but rather the direct effects of ethanol on calcium uptake appear to be overridden by the other changes associated with ethanol administration.

In summary, the present results indicate that ethanol acts primarily on the voltage-dependent calcium channel, and that the direct neuronal effect of chronic ethanol exposure is an increase in both calcium channel density and sensitivity to *in vitro* ethanol.

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## Catecholamine-metabolizing enzyme activity in the nigrostriatal system

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The nigrostriatal system is a well-defined model for the central catecholaminergic pathway [1]. This system has been studied extensively with respect to the synthesis, release, action, and degradation of dopamine. Lesion of either the presynaptic or postsynaptic elements has been employed to localize various enzymes related to catecholamine metabolism.

With respect to monoamine oxidase (MAO), several studies have shown that the high striatal activity of this enzyme remains unaffected after damage to the presynaptic

elements by direct nigral lesions or by application of 6-hydroxy-dopamine [2–7]. These initial studies measured MAO activity with substrates that do not discriminate between MAO A and B, both of which are present in rat striatum. With more selective substrates, a specific loss in MAO A activity with no change in MAO B activity has been noted [7, 8]. The stability of striatal MAO B to these presynaptic lesions is consistent with the localization of this species to glial cells [9–11]. A more recent report, however, was unable to confirm the loss of MAO A activity in the striatum upon unilateral electrolytic lesioning of the substantia nigra [12].

Catechol-*O*-methyltransferase (COMT) is a catecholamine-metabolizing enzyme whose activity has been studied by presynaptic lesions of both striatum and cerebral cortex. These studies have uniformly shown no loss of COMT activity after such lesions [4, 7, 13] and have been viewed as consistent with the immunohistochemical localization of COMT to glial cells [14]. Unfortunately, these earlier papers on localization of COMT activity did not distinguish between the soluble (SOL-COMT) and membrane-bound (MB-COMT) form which differ markedly in their $K_m$ values for dopamine [15]. The distinct membrane-bound species of COMT has recently been demonstrated to be localized in the neurons intrinsic to the striatum [16].

A third enzyme in rat brain capable of metabolizing catecholamines and with a high specific activity in the striatum is the dopamine-preferring (Type IV) form of phenol sulfotransferase [17]. Data from kainic-acid lesions of the striatum indicate that this enzyme is present in the neurons intrinsic to the striatum [18]. Whether this enzyme is also localized in the presynaptic elements of the striatum has not been investigated.

The present study addresses localization of these enzymes in the striatum by measuring their activities in striatal homogenates prepared after electrolytic lesioning of the substantia nigra. The extent and specificity of the lesions were monitored by parallel estimates of marker enzyme activities: dopa decarboxylase (DDC, L-aromatic amino acid decarboxylase) with a predominantly presynaptic localization [19]; and glutamic acid decarboxylase (GAD), a postsynaptic neuronal enzyme [20].

*Materials and methods*

For the electrolytic lesions, male Sprague–Dawley rats (Blue Spruce Farms) were anesthetized with pentobarbital (42 mg/kg). An 0.75-mm diameter electrode insulated except for 0.75 mm at the tip was placed in the rostral substantia nigra (AP- 3.0 L 2.4, V 8.8), and current was passed with a Radionics RFG4 radiofrequency generator to achieve an electrode tip temperature of 85–90°. Sham-operated animals were subjected to placement of the electrode to V 6.0 without passage of current.

Nine days after lesioning the rats were killed by cervical dislocation, and the right and left striata were excised and homogenized by hand-held Teflon–glass tissue grinders in 45–55 vol. of cold 10 mM potassium phosphate buffer (pH 7.4). Aliquots of the homogenate were taken for protein determination [21]. COMT activity was estimated with [$^3$H]dopamine at 4 $\mu$M (favoring analysis of the MB-COMT) and 100 $\mu$M (favoring analysis of the SOL-COMT) by the method of Rivett *et al.* [15]. An estimated $V_{max}$ for each species was determined as described previously [16]. MAO activity was measured by the method of Roth and Feor [22] using $^{14}$C-labeled 5-hydroxytryptamine (100 $\mu$M) and phenylethylamine (10 $\mu$M) as selective substrates for the A and B forms of MAO respectively [8]. Sulfotransferase (PST) activity was determined by the Ecteola cellulose assay described by Whittemore and Roth [23] using 200 $\mu$M dopamine and 1 $\mu$M [$^{35}$S]3′-phosphoadenosine-5′-phosphosulfate as co-substrates. Aliquots of the homogenate were adjusted to 0.5% Triton X-100 for estimation of GAD [24] and DDC [25] activities by $^{14}CO_2$ trapping methods. Aliquots of the homogenate were stored at −20° for later assay of glutamine synthetase (GS; an astroglial selective marker enzyme) activity as described previously [11].

Striata from sham-operated animals did not differ from left or right striata of unoperated rats on any measurement. These data were pooled for control values. Comparisons were made by *t*-test.

Reagents and substrates were obtained from the Sigma Chemical Co., St. Louis, MO. Radiochemicals noted above were obtained from New England Nuclear, Boston, MA.

*Results and discussion*

Enzyme activities measured in homogenates prepared from rat striata after electrolytic lesioning are listed in Table 1. The significant loss of DDC activity with no loss in GAD activity or protein content indicates that the lesions were appropriately presynaptic with no postsynaptic component. The small increase in GAD activity observed after nigral lesioning has also been reported previously [26]. The increase in striatal GS activity post-lesion may reflect increased astrocytic hypertrophy or hyperplasia, since GS is strictly localized to astrocytes [27]. GS activity increases after kainic acid injections into striatum [16] which are accompanied by prominent astrocytic proliferation. Striatal

Table 1. Striatal activities 9 days after electrolytic lesion of substantia nigra

| Activity | Control | Lesion | % L/C | P |
|---|---|---|---|---|
| Protein content* | 11.8 ± 0.1 | 11.7 ± 0.1 | 99 | NS† |
| GAD† | 4.66 ± 0.20 | 5.80 ± 0.11 | 124 | 0.005 |
| DDC§ | 244 ± 8 | 32 ± 7 | 13 | 0.001 |
| GS† | 22 ± 1 | 27 ± 1 | 128 | 0.01 |
| PST§ | 3.34 ± 0.05 | 2.73 ± 0.01 | 82 | 0.001 |
| SOL-COMT‡ | 7.96 ± 0.66 | 9.04 ± 0.53 | 113 | NS |
| MB-COMT† | 0.16 ± 0.01 | 0.15 ± 0.01 | 94 | NS |
| MAO A§ | 0.89 ± 0.04 | 0.93 ± 0.08 | 104 | NS |
| MAO B§ | 4.84 ± 0.03 | 5.66 ± 0.14 | 117 | 0.001 |

Values are means ± SE; N = 4 for control and N = 6 for lesion. Abbreviations: GAD, glutamic acid decarboxylase; DDC, dopa decarboxylase; GS, glutamine synthetase; PST, phenol sulfotransferase; SOL-COMT, soluble catechol-*O*-methyltransferase; MB-COMT, membrane-bound catechol-*O*-methyltransferase; and MAO, monoamine oxidases.

* Expressed as percent of wet weight.

† NS, not significant.

‡ Expressed as nmol/min/mg protein.

§ Expressed as pmol/min/mg protein.

GS has apparently not been previously measured after presynaptic lesions, although histological studies have shown striatal astrocytic proliferation after nigrostriatal lesions in the rat [25, 28]. Consistent with the interpretation of striatal astrocytosis is the increased activity of MAO B and SOL-COMT in the deafferented striatum; activity of these species also increases in the kianic-acid-lesioned striatum as astrocytic proliferation develops [10, 11, 16]. The activity of MB-COMT, which has been shown to be present in the post-synaptic neurons of the striatum [16], was not altered by the electrolytic lesion, suggesting that this form of the methyltransferase is not present in the presynaptic terminals.

Dopaminergic nerve endings have been estimated to represent approximately 10–20% of striatal synaptic complexes [29]. Changes in enzyme specific activities as determined in homogenates would imply either that the activities were highly concentrated in the lesioned nerve endings (compared to the rest of striatal structures) or that the lesion induced a widespread secondary change in other striatal elements. Thus, the major finding of this study, that striatal PST was significantly decreased approximately 20% (Table 1) after nigral lesions, would suggest that this enzyme is relatively concentrated in the lesioned nerve endings. Previous studies have suggested a (postsynaptic) neuronal localization for PST with no increase during astrocytic proliferation, based on kainic-acid lesions [18]. Thus, it appears that the dopamine-metabolizing form of PST is present in both pre- and postsynaptic neurons of the nigrostriatal pathway.

Much controversy surrounds the interpretation of lesion studies aimed at localizing MAO activity. Immunohistochemical [9, 30] evidence indicates that MAO B is localized in astrocytes of striatum and may also be present in serotonergic neurons. The present data (Table 1) demonstrate a slight increase in striatal MAO B activity in striata after nigral lesions. The only prior study which addressed this question with a MAO B-selective substrate is that of Demarest *et al.* [8] who found no change in MAO B specific activity of a crude synaptosomal (P2) fraction 2 weeks after 6-hydroxy-dopamine injections into the substantia nigra. In the present study, the increased MAO B activity is consistent with, and parallels the other biochemical indices of, increased astrocytic activity (i.e. GS, SOL-COMT). The failure of Demarest *et al.* [8] to find a change in MAO B may reflect their use of tissue fractionation which is known to yield differential recoveries of MAO A versus B in normal striatum [31], and this differential recovery cannot be predicted in lesioned striatum.

The present data showing no effect of the lesions on striatal MAO A are consistent with studies employing nonselective substrates and confirm the data of VanderKrogt *et al.* [12]. The data again apparently contrast with that of Demarest *et al.* [8] who reported a 27% loss of MAO A in the striatal P2 fraction after 6-hydroxy-dopamine lesions. If, as might be expected, the P2 fraction contains about 70% of the total striatal MAO A, then the present data should show about a 20% loss in striatal MAO A as measured in the homogenates if the lesions were similar in degree. This change is within the sensitivity of the present methods as well as those of VanderKrogt *et al.* [12] who were also unable to replicate a loss of MAO A activity in either homogenates or in P2 fractions of striata after nigral 6-hydroxy-dopamine lesions. Although MAO A has been shown to be exclusively associated with catecholamine-containing neurons in primate brain, this has not been confirmed for rat brain.

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