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# Long-chain fatty acids increase cellular dopamine in an immortalized cell line (MN9D) derived from mouse mesencephalon

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**Abstract**

The lysate of an immortalized monoclonal cell line derived from the striatum (X61) contains a dopaminergic stimulatory activity that is capable of increasing the dopamine content of an immortalized mouse mesencephalic cell line (MN9D) which expresses a dopaminergic phenotype. Purification of an isoamyl alcohol extract of this lysate and subsequent identification by NMR spectroscopic analysis demonstrated that the dopaminergic stimulatory activity contained within the lysate was a mixture of 80–90% *cis*-9-octadecenoic acid (oleic acid) and 10–20% *cis*-11-octadecenoic acid (*cis*-vaccenic acid). The effect of oleic acid on MN9D dopamine is a prolonged event. MN9D dopamine increases linearly over a 48 h period suggesting the induction of an increased dopaminergic phenotype in these dividing cells. The ability to increase MN9D dopamine by oleic and *cis*-vaccenic acids is shared by a number of other long-chain fatty acids including arachidonic, linoleic, linolenic, palmitoleic, and *cis*-13-octadecenoic acid. The possibility that oleic or other relatively innocuous fatty acids might affect dopaminergic function in primary neurons is intriguing with respect to possible therapeutic approaches to the treatment of dopaminergic cell loss and the motor sequelae of Parkinson's disease.
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Immortalized monoclonal cells of the mouse nigrostriatal projection have been developed as an approach to the identification of substances which could regulate dopaminergic function and cell survival [1,14]. The dopaminergic MN9D cell line of mesencephalic origin and the X61 cell line of striatal origin were obtained by somatic cell fusion with the N18TG2 neuroblastoma which is lacking the hypoxanthine phosphoribosyltransferase enzyme [1,14]. We have previously demonstrated that striatal-derived monoclonal cells (X61) contain dopaminergic stimulatory substances which increase the dopamine content of MN9D cells [3]. Striatal cell lines (X61) provide a source for such substances and the mesencephalic-derived MN9D cell line provides a rapid assay method for detecting active molecules capable of modulating cellular dopamine. The crude cell lysate of X61 cells, as well as a partially purified ultrafiltrate preparation (UF4) of that lysate, also increases the dopamine content of primary dopaminergic neurons grown in reaggregate culture in the absence of target cells (i.e., mesencephalic cells co-cultured with tectum, a non-target region for dopaminergic neurons) as well as levels of homovanillic acid in the culture medium [15]. In such cultures, in which the majority of dopaminergic neurons are lost due to the absence of target cells, treatment with the crude lysate or UF4 ultrafiltrate results in a 2- (UF4) to 2.9- (X61 lysate) fold increase in the density of dopaminergic neurons in the treated cultures [15].

The UF4 ultrafiltrate contains active substances, probably peptides, of low molecular weight and high water solubility. It was, however, apparent that the bulk (two-thirds) of

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dopaminergic stimulatory activity present in the processed X61 cells resided in a fraction which did not pass through a YM-5 ultrafiltration membrane and was lipid soluble. The present study was conducted to determine the chemical nature and activity of this major fraction.

The UF4 ultrafiltrate was obtained from sonicated lysates of X61 cells which were allowed to "autodigest" at room temperature for 2 days and concentrated by pressure filtration through an Amicon YM-5 membrane (5000 Da molecular weight cut-off). Some form of digestion occurs in this process as evidenced by the fact that 2 days of incubation at room temperature results in the conversion of some (approximately 30%) of the dopaminergic stimulatory activity in the X61 cell lysate from a high molecular weight form to a size that can pass through a YM-5 membrane (see [15] for details).

The low molecular weight UF4 ultrafiltrate fraction contained significant dopaminergic stimulatory activity as assessed by effects on MN9D cells. However, the majority of the activity from the "autodigested" X61 cell lysate did not pass through the YM-5 ultrafiltration membrane. Approximately two-thirds of the activity resided in the material remaining on the high molecular weight side of the Amicon YM-5 membrane and is referred to as "X61 concentrate". This X61 concentrate was subsequently extracted with 2 M NaCl followed by a 1:1 mixture of isoamyl alcohol/chloroform. The isoamyl alcohol/chloroform extract was shown to contain materials capable of increasing MN9D dopamine levels. This activity is not extractable from fresh X61 cell lysate, but appears to require the autodigestion step with time for the activity to become liberated from some cell component and be available for organic extraction.

The isoamyl alcohol/chloroform soluble stimulatory activity was taken up by a C18 reverse phase column from a mixture of 70% acetonitrile/30% (0.05% trifluoroacetic acid in water) and then eluted by a linear gradient from the mixture to 100% acetonitrile. The active fractions from the column showed some absorbance at 215 nm, but the bulk of absorbance was seen in fractions devoid of activity, suggesting that the reverse phase separation resulted in considerable purification of the activity. The active fractions from the reverse phase column were then applied to a Phenomenex 5 μm, 50 Å Phenogel gel filtration sizing column. The dopaminergic stimulatory activity eluted from the Phenogel column in 100% acetonitrile within a single absorbance peak. The Phenogel fractions containing dopaminergic stimulatory activity were subjected to mass spectrographic analysis. Two peaks of high intensity were observed with molecular weights of 283 and 565.

NMR spectroscopy demonstrated that the single elution peak from the Phenogel column contained two chemical moieties, the greater of which constituted approximately 80–90% of the material (Fig. 1). The 600 MHz $^{1}$H 1D NMR spectrum of the sample showed one major set of peaks and no significant minor peaks, suggesting a sample purity greater than 95%. The $^{13}$C 1D NMR spectrum, however, showed two sets of peaks, indicating that the sample contained two species

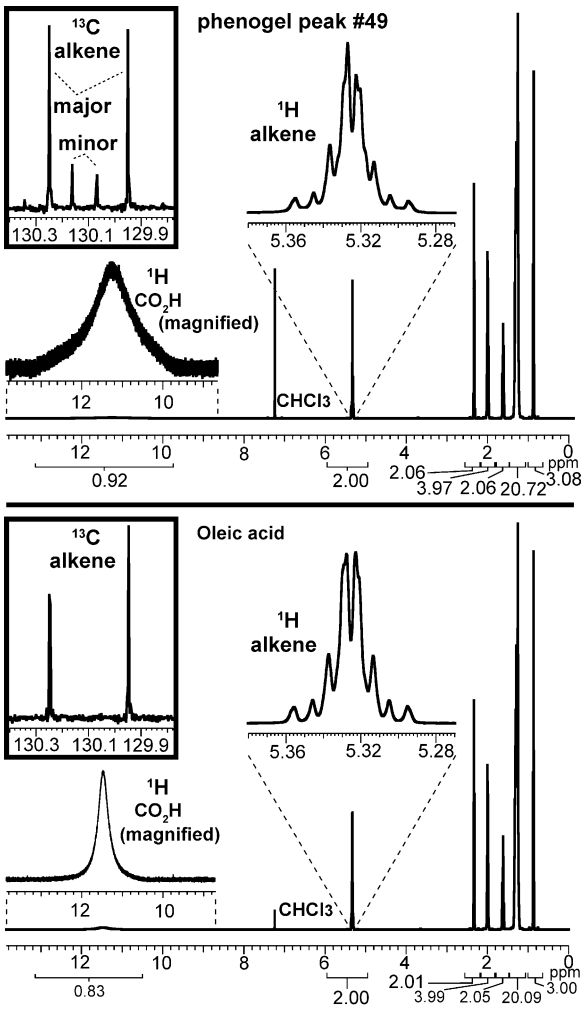


Fig. 1. The NMR spectra of peak #49 from the Phenogel column purified material (top) as compared to commercial oleic acid (bottom). The $^{1}$H spectrum is shown at the bottom of each panel, and the peak integral values of the intensities are indicated below the axes. The carboxylic acid and alkene regions of the $^{1}$H spectra are magnified and expanded. The alkene regions of the $^{13}$C spectra are shown as insets. It is apparent that the Phenogel #49 sample contains one major and one minor species. The minor species was identified by NMR analysis to be *cis*-vaccenic acid.

so closely related that they gave rise to virtually identical $^{1}$H NMR spectra. The minor component made up approximately 10–20% of the total sample, according to their relative intensities in the $^{13}$C spectra. The positions of the $^{1}$H and $^{13}$C peaks in these spectra ruled out the possibility that the sample contains protein, peptide, DNA, RNA, carbohydrate, glycolipid, steroid, or other cholesterol-related molecules. The positions are instead consistent with those expected from a fatty acid. Comparison of the major species' spectra with $^{1}$H and $^{13}$C NMR spectra of various fatty acids showed that they were very similar to those of *cis*-9-octadecenoic acid (oleic acid). It remained uncertain whether the unsaturated bond was of *cis* or *trans* configuration. Pure *cis*-9-octadecenoic acid and *trans*-9-octadecenoic acid (elaidic acid) (Aldrich), and their $^{1}$H and $^{13}$C NMR spectra were compared to those of the sam-

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