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31 August 1990; 29 October 1990

D_1 and D_2 Dopamine Receptor–Regulated Gene Expression of Striatonigral and Striatopallidal Neurons

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The striatum, which is the major component of the basal ganglia in the brain, is regulated in part by dopaminergic input from the substantia nigra. Severe movement disorders result from the loss of striatal dopamine in patients with Parkinson's disease. Rats with lesions of the nigrostriatal dopamine pathway caused by 6-hydroxydopamine (6-OHDA) serve as a model for Parkinson's disease and show alterations in gene expression in the two major output systems of the striatum to the globus pallidus and substantia nigra. Striatopallidal neurons show a 6-OHDA–induced elevation in their specific expression of messenger RNAs (mRNAs) encoding the D_2 dopamine receptor and enkephalin, which is reversed by subsequent continuous treatment with the D_2 agonist quinpirole. Conversely, striatonigral neurons show a 6-OHDA–induced reduction in their specific expression of mRNAs encoding the D_1 dopamine receptor and substance P, which is reversed by subsequent daily injections of the D_1 agonist SKF-38393. This treatment also increases dynorphin mRNA in striatonigral neurons. Thus, the differential effects of dopamine on striatonigral and striatopallidal neurons are mediated by their specific expression of D_1 and D_2 dopamine receptor subtypes, respectively.

THE BASAL GANGLIA ARE A MAJOR brain system through which the cerebral cortex affects behavior. Processing of cortical input in the striatal portion of the basal ganglia is modulated by dopaminergic input from the substantia nigra (*1*). This dopaminergic modulation has profound effects on behavior. Reduction in striatal dopamine levels, as in Parkinson's disease, results in reduced movement (*2*), whereas excessive dopaminergic action within the striatum produces involuntary dyskinetic movements (*1*). Dopamine differentially regulates the two major output pathways from the striatum, one to the globus pallidus (the external segment of the globus pallidus in primates) and the other to the substantia nigra and entopeduncular nucleus (the internal segment of the globus pallidus in primates) (*1, 3*). These output pathways arise from the major postsynaptic target of dopamine in the striatum, the medium spiny neurons (*4*), which constitute more than 90% of the striatal neuronal population. Although these neurons often provide collaterals to more than one nucleus, the target of the main axon collateral distinguishes two subpopulations of approximately equal numbers of striatopallidal neurons and striatonigral neurons (the entopeduncular nucleus and substantia nigra are here considered part of the same extended nuclear complex) (*5*). All of the medium spiny neurons utilize γ-aminobutyric acid (GABA) as a neurotransmitter (*6*), but the two subpopulations contain different neuropeptides. Striatopallidal neurons contain enkephalin, whereas striatonigral neurons contain substance P and dynorphin (*7, 8*). These peptides are differentially regulated by dopamine. Reduction of dopamine input to the striatum results in increased expression of enkephalin (*9, 10*) and a reduction in substance P (*11*). Conversely, increases in dopamine receptor stimulation result in increases in both substance P and dynorphin levels (*12*). These actions of dopamine may be differentially mediated by D_2 and D_1 dopamine receptors (*13*). Both D_1 and D_2 dopamine receptors act via G proteins; D_1 receptors activate and D_2 receptors inhibit adenylyl cyclase activity in striatal neurons (*14*). Indirect evidence suggests that D_1 and D_2 dopamine receptors are differentially expressed in striatonigral and striatopallidal neurons (*15*).

In a first experiment, striatonigral and striatopallidal neurons were characterized by their expression of D_1 and D_2 dopamine receptor subtypes and the peptides dynorphin, substance P, and enkephalin with in situ hybridization histochemistry (ISHH) combined with retrograde labeling of striatonigral neurons (Fig. 1). Oligonucleotide probes (48 bases in length) complementary to the mRNA encoding the D_1 and D_2 dopamine receptors and the peptides dynorphin, substance P, and enkephalin (*16*) were labeled with ^{35}S-labeled deoxyadenosine monophosphate (dAMP) tails of approximately 25 bases in length (*17*) and used for ISHH labeling of striatal sections from animals that had received an injection of fluorogold into the substantia nigra (*18*). Approximately 43% of the striatal cells were retrogradely labeled with fluorogold (194 of 452 cells). Fluorogold-labeled striatonigral neurons were often labeled with the D_1 probe (149 of 173 D_1 cells), the dynorphin

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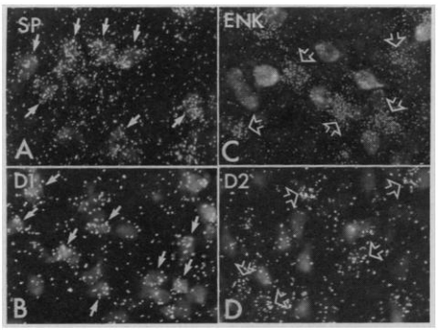

Fig. 1. Striatal neurons retrogradely labeled with the fluorescent dye fluorogold after injection into the substantia nigra combined with darkfield illumination of silver grains produced by ISHH labeling with ^{35}S-labeled oligonucleotide probes for (**A**) substance P (SP), (**B**) the D1 dopamine receptor (D1), (**C**) enkephalin (ENK), and (**D**) the D_2 dopamine receptor (D2). Striatonigral neurons show ISHH labeling for both substance P [(A), solid arrows] and the D_1 dopamine receptor [(B), solid arrows]. Striatal neurons that are unlabeled by fluorogold, and presumably project to the globus pallidus, show ISHH labeling for both enkephalin [(C), open arrows] and the D_2 dopamine receptor [(D), open arrows].

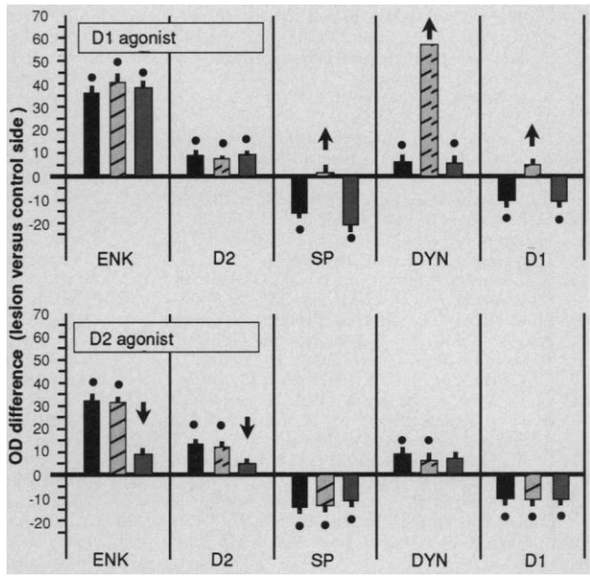

Fig. 3. The average difference (±SEM) in ISHH labeling between the 6-OHDA–injected (lesioned) and unlesioned striatum as measured in optical density units (OD) for each group of animals (n = 6 for each group). Relative changes in ISHH labeling for enkephalin (ENK), the D_2 dopamine receptor (D_2), substance P (SP), dynorphin (DYN) and the D_1 dopamine receptor (D_1) are plotted for groups receiving the D_1 agonist SKF-38393 (top) and the D_2 agonist quinpirole (bottom) by either single daily injections for 21 days (intermittent) or via an osmotic minipump planted intraperitoneally (continuous) and compared to saline treatment (saline controls). Dots, significant differences determined with a two-way analysis of variance and a Newman-Keuls post-hoc analysis (P < 0.01). Significant changes in the lesion-induced effects in drug-treated groups are marked with arrows indicating the direction of change (P< 0.001).

probe (97 of 118 dynorphin cells), and the substance P probe (103 of 145 substance P cells). Conversely, fluorogold-labeled neurons were seldom labeled with the D_2 dopamine receptor probe (27 of 184 D_2 cells) or the enkephalin probe (32 of 187 enkephalin cells). It is assumed that the majority of fluorogold-negative neurons that contain enkephalin mRNA (155 cells) and D_2 mRNA (157 cells) are striatopallidal neurons (*8*). Although there may be a small percentage of striatal neurons that express both receptors, our data suggest that the majority of striatonigral neurons express the D_1 dopamine receptor as well as the neuropeptides dynorphin and substance P, whereas the majority of striatopallidal neurons express both the D_2 dopamine receptor and enkephalin.

In a second experiment, we dissociated the effects of D_1- and D_2-mediated receptor regulation of striatal peptide expression by first making unilateral lesions of the nigrostriatal dopaminergic pathway with 6-OHDA followed by either repeated daily injections or continuous administration of D_1- and D_2-selective agonists. In adult male rats unilateral lesions of the nigrostriatal pathway were made by injection of 6-OHDA (4 μg in 2 μl of 0.01% ascorbate and 0.9% saline) into the substantia nigra. Twenty-one days later the efficacy of the 6-OHDA–caused lesions was assessed by examining apomorphine (0.05 mg/kg of body weight)–induced contralateral rotation. Animals that displayed contralateral rotation were divided into six groups (six animals per group) and treated for 21 days with one of the following drug schedules: (i) intermittent injections of the D_1 receptor–selective agonist SKF-38393 (once daily, 12.5 mg/kg, injected intraperitoneally), (ii) continuous infusion of SKF-38393 via an osmotic minipump (12.5 mg/kg per day, implanted intraperitoneally), (iii) intermittent injections of the D_2 receptor–selective agonist quinpirole (once daily, 1 mg/kg, injected intraperitoneally), (iv) continuous infusion of quinpirole with a minipump (1 mg/kg per day, implanted intraperitoneally), and (v) vehicle injections and infusions for 21 days (two control groups). All of the animals were killed 3 hours after the last injection; their brains were removed, frozen in isopentane (−70°C), and processed for ISHH labeling (*19*). After hybridization, the sections were apposed to x-ray film for 7 to 20 days, the film was developed, and the average optical density (OD) over the striatum on the unlesioned and lesioned side of each section was measured (Fig. 2).

We confirmed that the nigrostriatal pathway was lesioned by noting a greater than 90% loss of dopamine neurons in the substantia nigra pars compacta labeled by ISHH with a tyrosine hydroxylase oligonucleotide probe. None of the drug treatments resulted in a significant alteration of the amounts of peptide mRNAs on the unlesioned side when compared with the amounts in saline-treated animals, as seen from a two-way analysis of variance and the Newman-Keuls post-hoc analysis. Significant differences (p < 0.01) were seen in the

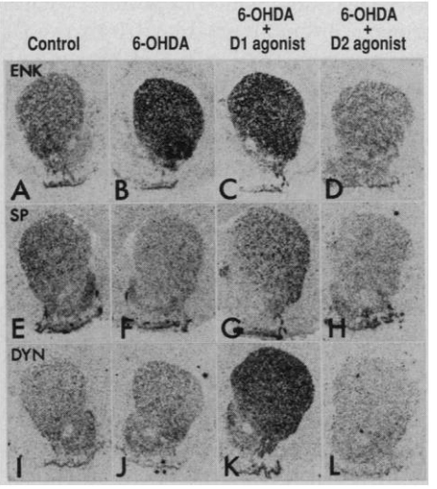

Fig. 2. In situ hybridization in the striatum from brain sections apposed to autoradiographic film with ^{35}S-labeled oligonucleotide probes complementary to (**A** through **D**) enkephalin (ENK), (E through H) substance P (SP), and (I through L) dynorphin (DYN). Sections in the first two columns are from the same saline-treated animal showing ISHH labeling on the unlesioned control side (**E** and **I**) and lesioned 6-OHDA–injected side (**B**, **F**, and **J**). Sections in the third column are from the lesioned, 6-OHDA–injected side of an animal that received intermittent treatment with the D_1 agonist SKF-38393 (**C**, **G**, and **K**). Sections in the fourth column are from the lesioned, 6-OHDA–injected lesioned side of an animal that received continuous treatment with the D_2 agonist quinpirole (**D**, **H**, and **L**). The increase in enkephalin ISHH labeling caused by 6-OHDA lesions (B) is not affected by D_1 agonist treatment (C) but is reversed by continuous D_2 agonist treatment (D). The decrease in substance P ISHH labeling caused by 6-OHDA lesions (F) is reversed by intermittent D_1 agonist treatment (G) but is unaffected by D_2 agonist treatment (H). Dynorphin ISHH labeling is not significantly altered by 6-OHDA lesions (J) but is elevated by D_1 agonist treatment (K) but not affected by D_2 agonist treatment (L).

levels of ISHH labeling in the lesioned striatum for the different experimental groups (Fig. 3). In saline-treated animals, ISHH labeling for enkephalin and the D_2 receptor was significantly elevated in the lesioned striatum. These lesion-induced increases in mRNA expression were specifically reversed by continuous administration of the D_2-selective agonist quinpirole. None of the other drug treatments, intermittent quinpirole or either SKF-38393 treatment, significantly altered the lesion-induced elevation of enkephalin or D_2 receptor ISHH labeling. In saline-treated animals, ISHH labeling for substance P and the D_1 receptor was significantly reduced in the lesioned striatum. These lesion-induced decreases were reversed by intermittent treatment with the D_1-selective agonist SKF-38393. Dynorphin ISHH labeling in the striatum, which was not significantly affected by the lesion, was markedly increased with intermittent injections of the D_1-selective agonist SKF-38393 for 21 days. Continuous treatment with SKF-38393 or treatment with quinpirole by either intermittent or continuous regimens did not significantly affect the lesion-induced changes in dynorphin, substance P, or D_1 receptor ISHH levels.

The significance of D_1 and D_2 dopamine receptor–specific regulation of striatonigral and striatopallidal pathways is related to their opposite effect on GABAergic neurons in the entopeduncular nucleus and substantia nigra pars reticulata as diagrammed in Fig. 4. These neurons provide tonic inhibitory inputs to brain areas that directly generate movements. For example, phasic pauses in the tonic activity of pars reticulata neurons disinhibit target neurons in the superior colliculus, generating eye movements (*20, 21*). Inhibition of the tonic activity of pars reticulata neurons is produced by striatonigral neurons which are excited by cortical or thalamic (or both) glutamatergic neurons (*21*). Conversely, excitation of striatopallidal neurons, which inhibits the tonic activity of GABAergic neurons in the globus pallidus and results in a disinhibition of the excitatory subthalamic input to the substantia nigra, results in increased tonic activity of pars reticulata neurons (*22*). Thus, normal movement results from a coordinated balance of cortical and thalamic excitation of the striatonigral and striatopallidal pathways that regulate the tonic activity of substantia nigra pars reticulata neurons. Dopamine modulates this balance. Dopamine deafferentation in the striatum results in an increased output of the striatopallidal pathway (*23, 24*). The resulting increased activity of pars reticulata neurons results in the bradykinesia of Parkinson's disease (*1*). To some extent gene expression parallels the changes in activity in striatopallidal and striatonigral neurons; dopamine deafferentation results in increases in both enkephalin and D_2 receptor mRNA expression in striatopallidal neurons, whereas there is a decrease in substance P and D_1 receptor mRNA expression in striatonigral neurons.

Our data suggest that D_2 and D_1 agonist treatments selectively reverse the effects of dopamine depletion by way of the specific expression of D_2 and D_1 receptors on striatopallidal and striatonigral neurons, respectively. This does not rule out synergistic D_1 and D_2 actions in neurons that may express both receptors or by interactions among striatal interneurons, striatonigral neurons, and striatopallidal neurons. Our data also indicate the importance of the time course of drug delivery. Intermittent injections of a D_1 agonist appear necessary to produce regulation of striatonigral neurons, whereas continuous treatment with a D_2 agonist appears necessary to regulate striatopallidal neurons.

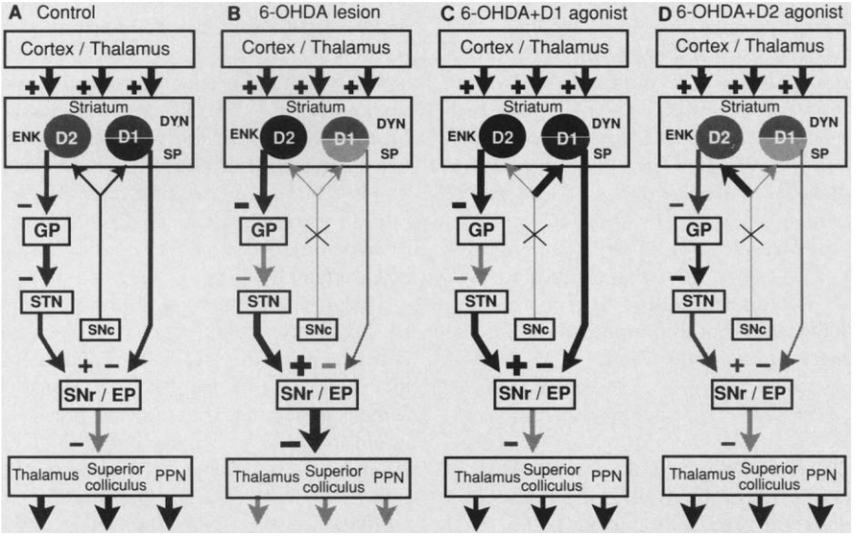

Fig. 4. Diagram of the connections of striatal output neurons. The degree of shading of the cells denotes relative peptide mRNA levels, and the thickness of the lines indicates relative activity measured in studies of 2-deoxyglucose measurements (*24*). (**A**) Control: The cortex and thalamus provide an excitatory input to the striatum. Striatal neurons that contain enkephalin (ENK) and the D_2 dopamine receptor provide an inhibitory input to the globus pallidus (GP). Pallidal neurons provide an inhibitory input to the subthalamic nucleus (STN), which provides an excitatory input to the substantia nigra pars reticulata (SNr). Striatal neurons that express the D_1 dopamine receptor, dynorphin (DYN) and substance P (SP) provide an inhibitory input to the substantia nigra/entopeduncular nucleus (SNr/EP). SNr/EP GABAergic neurons inhibit neurons in the thalamus, superior colliculus, and pedunculopontine nucleus (PPN). Normal behavioral activity (arrows at the bottom of the diagram) is dependent on coordinated striatonigral and striatopallidal outputs that regulate SN-output. (**B**) 6-OHDA: Dopamine lesions result in increased enkephalin expression and activity in striatopallidal neurons. This results in increased firing of SNr GABAergic neurons and in diminished behavioral activity (arrows at bottom of diagram). (**C**) 6-OHDA + D_1 agonist: D_1 agonist treatment after 6-OHDA lesions does not alter the lesion-induced increase in enkephalin in the striatopallidal pathway but reverses the decrease in substance P and significantly increases dynorphin in striatonigral neurons. (**D**) 6-OHDA + D_2 agonist: Continuous D_2 agonist treatment after 6-OHDA lesions has no effect on the striatonigral pathway but reverses the lesioned-induced increase in enkephalin in the striatopallidal pathway. This reverses the increased excitatory input from the subthalamic nucleus to the substantia nigra pars reticulata.

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16. Oligonucleotide probes used for localization of enkephalin, dynorphin, and substance P have been described (*8, 9*). The D_2 dopamine receptor probe sequence was derived from amino acids 10 to 25 of a D_2 receptor, F. J. Monsma, Jr., L. C. Mahan, L. D. McVittie, C. R. Gerfen, D. R. Sibley, *Nature* **342**, 926 (1989). The D_1 dopamine receptor probe sequence was derived from amino acids 277 to 289 of a D_1 dopamine receptor first derived from a mouse neuroblastoma cell line, F. J. Monsma, Jr., L. C. Mahan, L. D. McVittie, C. R. Gerfen, D. R. Sibley, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6723 (1990).
17. Tails of ^{35}S-labeled dAMP (average tail length of 20 bases) were added to purified oligonucleotides (48-base cDNAs) with terminal deoxynucleotidyl transferase (TdT, Bethesda Research Labs, Gaithersburg, MD) according to the method of Young *et al.* (*9*).
18. The fluorescent retrograde axonal tracer fluorogold [L. C. Shmued and J. H. Fallon, *Brain Res.* **377**, 147 (1986)] was injected into the substantia nigra of anesthetized adult Sprague-Dawley rats. After 5 days, the animals were anesthetized and perfused transcardially with saline followed by 4% formaldehyde in 0.1 M sodium phosphate–buffered saline (pH 7.4). The brains were removed and processed as described for ISHH labeling (*8, 9*).
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25. We would like to thank M. Brownstein for providing the oligonucleotide probes used in these experiments and for his invaluable advice and support.

5 July 1990; accepted 24 September 1990

Technical Comments

Formation of Ozone by Irradiation of Oxygen at 248 Nanometers

Slanger *et al.* (*1*) report that 248-nm KrF laser radiation produces ozone from oxygen, even though this wavelength is longer than 242.4 nm, the conventionally accepted threshold for the photodissociation of the ground electronic state $X^3\Sigma_g^-$ of O_2 (*2*). Slanger *et al.* (*1*) have demonstrated that the production of O_3 is initially inefficient and slow, but subsequently becomes efficient and rapid, in an autocatalytic fashion. They have shown that the latter efficient mechanism involves the 248-nm photodissociation of previously formed O_3 to yield atomic oxygen and $O_2(X^3\Sigma_g^-)$ in a range of vibrational levels including $v'' = 7,9$; these two levels then absorb the 248-nm radiation, forming $O_2(B^3\Sigma_u^-)$ ($v' = 2,7$), which is known (*3*) to predissociate, efficiently yielding a pair of ground-state oxygen atoms. The atomic oxygen formed directly from the photodissociation of O_3 and indirectly from the predissociation of $O_2(B^3\Sigma_u^-)$ is available to produce more O_3.

The initiating mechanism for the formation of O_3 from O_2 at 248 nm, however, remains somewhat obscure. Slanger *et al.* (*1*) make brief mention of three possibilities for this initial, inefficient step: dissociation of O_2 by two-photon absorption; dissociation of O_2 by radiation of wavelength ~239 nm arising from the anti-Stokes Raman shifting of the 248-nm radiation; and dissociation of $(O_2)_2$ dimers at 248 nm. The first and second suggestions seem unlikely with the unfocused radiation used, and no convincing evidence exists for the third process, in so far as the continuous attenuation of radiation incident on oxygen at 1 atm pressure at wavelength greater than 242.4 nm is mainly due to Rayleigh scattering. Slanger *et al.* (*1*) conclude that the initial formation of O_3 occurred in some undefined manner.

We suggest that a highly probable initiating step is the absorption of the 248-nm radiation by $O_2(X^3\Sigma_g^-)$ ($v'' = 1$), of which the fractional Boltzmann population at 300 K is ~0.057%, The photodissociation threshold for absorption into the Herzberg continuum $O_2(A^3\Sigma_u^+)$ by $O_2(X^3\Sigma_g^-)$ ($v'' = 1$) is 251.9 nm, compared with 242.4 nm for $O_2(X^3\Sigma_g^-)$ ($v'' = 0$), so that the 248-nm laser radiation can photodissociate the former but not the latter. The importance of the absorption of radiation by O_2 ($v'' = 1$), even at a concentration ~0.057% at 300 K, is well established in other spectral regions (*3, 4*).

In view of the facts (i) that the Franck-Condon factor for the (11,1) Herzberg I absorption band at 252.5 nm is ~5 times greater than that of the (11,0) band at 243.0 nm (*5*), and (ii) that the cross section for absorption from $v'' = 0$ into the Herzberg continuum near 242 nm is $\sim 1.0 \times 10^{-24}$ cm^2 (*2*), we estimate that the cross section for absorption of 248-nm radiation from $v'' = 1$ into the Herzberg continuum is $\sim 5 \times 10^{-24}$ cm^2. This estimation ignores the additional pressure-dependent cross section (*2*), which, at 1000 torr (the pressure of the experiment of Slanger *et al.*), could contribute an extra 2.7×10^{-24} cm^2, if we assume the pressure dependence for absorption by $O_2(v'' = 1)$ at 248 nm is the same as that for O_2 ($v'' = 0$) at 242 nm. In the experiment of Slanger *et al.* (*1*), a single laser pulse of 145 mJ corresponds to $\sim 1.8 \times 10^{17}$ photons, so that at the stated 10 Hz repetition rate $\sim 9.0 \times 10^{15}$ photons cm^{-3}s^{-1} are injected into the 200 cm^3 cell. Corresponding to the cell length of 15 cm and, for the $O_2(v'' = 1)$, a partial pressure of 0.6 torr and our estimated absorption cross section of $\sim 5 \times 10^{-24}$ cm^2, the optical depth in I_0/I is $\sim 1.4 \times 10^{-6}$, so that 1.3×10^{10} photons cm^{-3}s^{-1} are absorbed by O_2 ($v'' = 1$) and produce 2.6×10^{10} O_3 molecules cm^{-3}s^{-1}. After 10 min, a typical time for equilibrium to be reached (*1*), 1.6×10^{13} O_3 molecules cm^{-3} would have been produced, compared with O_3 densities of $\sim 1 \times 10^{16}$ cm^{-3} observed by Slanger *et al.* Thus, the process described, involving the absorption of 248-nm radiation by O_2 ($v'' = 1$), seems acceptable as the seeding mechanism that initiates the autocatalytic production of O_3 referred to in the first paragraph.

The proposed initiating mechanism could be tested by measuring the temperature dependence of the rate of formation of O_3 in experiments of the type performed by Slanger *et al.*, but with the cell previously baked in vacuo to achieve outgassing. For example, the fractional Boltzmann population of O_2 ($v'' = 1$) is ~1.1% at 500 K, ~0.06% at 300 K, and ~0.001% at 195 K. Slanger *et al.* noted that warming the cell walls resulted in a marked lowering of the O_3 concentration and attribute this to desorbed water vapor, which is then attacked by O^1D (from the photolysis of O_3 at 248 nm) to form OH, which catalytically destroys O_3. This effect, which is not related to the issue of the initiating mechanism, would be eliminated by prior outgassing of the cell.

The formation of O_3 by irradiation of O_2 at 210 nm (*1*) and 214 nm (*6*) has been observed. The production of O_3 at these wavelengths increases linearly with time and is not autocatalytic in nature. These wavelengths are shorter than 242.4 nm, the