D_1 Dopamine Receptor Activation Required for Postsynaptic Expression of D_2 Agonist Effects

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D_1 and D_2 dopamine receptors exert synergistic effects on the firing rates of basal ganglia neurons and on the expression of stereotyped behavior in rats. Moreover, the ability of D_2 agonists to induce changes in basal ganglia single unit activity and spontaneous motor activity is dependent upon the presence of endogenous dopamine to stimulate D_1 receptors; in rats treated with α-methyl-*p*-tyrosine to reduce endogenous dopamine levels, the neurophysiological and behavioral effects of the D_2 agonist quinpirole are significantly attenuated, while the effects of nonselective agonists like apomorphine, which stimulate both D_1 and D_2 receptors, or combinations of a D_2 agonist and a D_1 agonist are not attenuated. Thus, the previously held view that D_2 receptors alone are responsible for evoking the changes in behavior and basal ganglia output induced by nonselective dopamine agonists and endogenous dopamine is not supported by these results, which indicate that these phenomena require concurrent stimulation of both dopamine receptor subtypes.

SINCE KEBABIAN AND CALNE (*1*) first presented evidence for two dopamine receptor subtypes, D_1 and D_2, considerable attention has been directed toward understanding the relative contributions of these receptor subtypes to the mediation of physiological and behavioral phenomena induced by dopamine and its agonists. For years, little function was ascribed to the D_1 dopamine receptors in the central nervous system (CNS) beyond their ability to stimulate dopamine-sensitive adenylate cyclase. D_2 receptors of the CNS have been considered responsible for eliciting the behavioral sequelae and certain of the biochemical alterations attending the administration of classical nonselective dopamine agonists such as apomorphine (*2*) that stimulate both receptor subtypes. However, several studies have raised questions about the presumed independence of the D_2 receptor in mediating such dopamine agonist–induced effects (*3–6*). We have therefore sought to determine whether processes evoked by stimulation of postsynaptic D_1 and D_2 receptors interact to elicit neurophysiological and behavioral effects like those induced by the nonselective dopamine agonists, and to determine whether such an interaction between receptor-mediated processes might in fact be necessary for the expression of these effects.

We examined the abilities of selective D_1 and D_2 agonists, administered alone and in combination, to induce apomorphine-like changes in the activity of output neurons of the basal ganglia and to elicit classical dopamine agonist–related behaviors. The results show that D_1 and D_2 receptors exert synergistic effects on the firing rates of basal ganglia neurons and on the expression of stereotypic behavior in rats. Moreover, concurrent stimulation of both receptor subtypes appears to be required to evoke the changes in behavior and basal ganglia output typically observed with the nonselective dopamine agonists. These results indicate that currently held concepts regarding the relative roles of the two dopamine receptor subtypes in the CNS may need to be modified.

Effects of dopamine receptor stimulation on basal ganglia neuronal output were studied by recording tonic extracellular single unit activity in the rat globus pallidus (*7*). The globus pallidus receives a major input from the striatum and thus activity in the globus pallidus is likely to reflect changes in the level of striatal dopamine receptor stimulation; it also receives a sparse but widespread innervation from dopamine cells located in the substantia nigra pars compacta (*8*). Previous studies have shown that nonselective dopamine agonists such as apomorphine and pergolide (*9*), which stimulate both D_1 and D_2 receptors, substantially increase the activity of cells in the globus pallidus (*10–13*). Similar changes are not induced by drugs that alter serotonin or norepinephrine receptor stimulation (*10, 12, 14*).

When administered systemically, the selective D_2 agonist quinpirole induced dose-dependent increases in pallidal neuron activity that were significantly smaller than those induced by the nonselective agonists, even though the efficacy and potency of quinpirole at inhibiting the activity of dopamine cells in the substantia nigra pars compacta via the dopamine D_2 autoreceptors are equal to that of apomorphine (*13*). When SKF 38393, a selective D_1 agonist, was administered before quinpirole, however, marked increases in pallidal neuron activity, indistinguishable from those induced by apomorphine, were observed. The inactive form of SKF 38393, S-SKF 38393 (*15*), did not potentiate the effects of quinpirole (*13*). Our results indicate that the synergistic effects of quinpirole and the D_1 agonist reflect a general property of dopamine D_1 and D_2 receptor function rather than a pharmacodynamic or idiosyncratic interaction between these two agonists; a second selective D_2 agonist, RU 24926, produced similar effects when given together with SKF 38393. RU 24926 (*16*) is structurally dissimilar to quinpirole (*17*) and is also equipotent with apomorphine in inhibiting the activity of substantia nigra dopamine cells (*13*). Administration of RU 24926 (0.3 mg per kilogram of body weight, intravenously) alone produced an average increase in pallidal neuron firing rates of 30 ± 9% (SEM, $n = 8$) (Figs. 1A and 2). SKF 38393, given alone (20 mg per kilogram of body weight, intravenously), produced an average rate increase of 17 ± 6% ($n = 43$) (Fig. 2). However, when RU 24926 was administered 15 minutes after SKF 38393, a significantly potentiated rate increase of 85 ± 17% ($n = 12$) was observed (Figs. 1B and 2). As with quinpirole, this increase is comparable to that induced by an equimolar dose of apomorphine (Figs. 1E and 2) or pergolide, greater than that observed with RU 24926 or SKF 38393 alone, and greater than the additive effects of SKF 38393 and RU 24926 given separately (*18*).

Behavioral studies of the effects of selective D_1 and D_2 receptor stimulation yield compatible results. Quinpirole (3 mg per kilogram of body weight, subcutaneously) significantly increased nonstereotypic locomotion, grooming, and sniffing in the normal animal, but failed to induce the intense, continuous stereotyped behavior typically observed with apomorphine (0.75 mg per kilogram of body weight, subcutaneously) (Table 1). Behaviors observed with this dose of quinpirole resemble those elicited by lower doses (in the range of 0.2 mg per kilogram of body weight, subcutaneously) of apomorphine (*19*). Moreover, even at high doses (48 mg per kilogram of body weight), quinpirole does not elicit intense licking, biting, gnawing, or repetitive head and limb

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patterned stereotypic behaviors (*20*). Similarly, SKF 38393 (20 mg per kilogram of body weight, subcutaneously) elicited intense grooming, but no stereotypic behavior. However, when quinpirole was administered simultaneously with SKF 38393, intense stereotypic licking, biting, and gnawing behavior was observed with a concomitant decrease in organized motor activities such as locomotion, grooming, and sniffing (Table 1). In addition, as in the electrophysiological studies, evidence of synergistic interactions between D_1 and D_2 receptors was observed in behavioral studies utilizing RU 24926 (3 mg per kilogram of body weight, subcutaneously). This second selective D_2 receptor agonist also induced no intense, focused licking, biting, or gnawing or head and limb patterned stereotypies, although nonstereotypic locomotion, grooming, and sniffing were observed. When SKF 38393 was administered simultaneously with RU 24926, however, intense stereotypy was induced; nonstereotypic grooming, sniffing, and locomotion were concomitantly reduced (Table 1).

The requirement for both D_1 and D_2 receptors to be stimulated to induce changes in behavior and tonic pallidal neuron activity comparable to those occurring with nonselective dopamine agonists could explain the puzzling observation that the selective D_1 antagonist SCH 23390 blocks the behavioral effects of apomorphine and amphetamine (*4*), as well as the finding that SCH 23390 attenuates the effects of apomorphine and *d*-amphetamine on pallidal cell activity (*12*). In fact, SCH 23390 is as potent a blocker of apomorphine's effects on pallidal activity as YM-09151-2, a D_2 antagonist (*21*) (Fig. 3). The inactive S enantiomer of the D_1 antagonist, SKF 83566 (0.1 mg per kilogram of body weight), the 8-bromo analogue of SCH 23390 (*22*), failed to attenuate the rate-increasing effects of apomorphine (0.3 mg per kilogram of body weight) on pallidal cell activity. These studies show that stereoselective D_1 receptor blockade, as well as D_2 receptor blockade, attenuate the effects of a nonselective dopamine agonist on globus pallidus neurons and further support the idea that apomorphine's effects in the basal ganglia require concurrent D_1 and D_2 receptor stimulation.

Our results raised another question: could the characteristic effects induced by a D_2 agonist depend on endogenous dopamine that was providing tonic D_1 receptor stimulation? The fact that SCH 23390 attenuated the effects of quinpirole on pallidal single unit activity (*12*) supports this view. In addition, our data (Table 1) demonstrate the attenuating effect of SCH 23390 on behaviors induced by quinpirole. The role of tonic endogenous D_1 receptor stimulation in the induction of neurophysiological and behavioral phenomena by D_2 agonists was therefore explored in animals in which endogenous dopamine levels were reduced before the experiment by α-methyl-*p*-tyrosine (AMPT) treatment (*23*). If the D_2 receptor independently mediates the effects of the D_2 agonist, then the full range of behavioral and neurophysiological effects occurring in intact animals should persist in such a preparation. In fact, the effect of a quinpirole dose (1 mg per kilogram of body weight, intravenously) that increases pallidal cell firing in the normal rat was significantly attenuated in rats pretreated with AMPT (Figs. 1D and 2). However, when SKF 38393 was administered after quinpirole to AMPT-treated rats, marked increases in pallidal neuron firing rates were observed equivalent to those induced by either apomorphine or by the combination of these agents in the intact animal (Fig. 1, E and F, and Fig. 2). Consistently, the effects of apomorphine, which is capable of stimulating both receptor subtypes, were not attenuated in AMPT-treated rats. Increases in pallidal neuron activity after apomorphine administration in this preparation did not significantly differ from the increases induced by apomorphine in the intact animal.

The behavioral effects of quinpirole and SKF 38393 in normal and catecholamine-depleted rats paralleled these neurophysio-

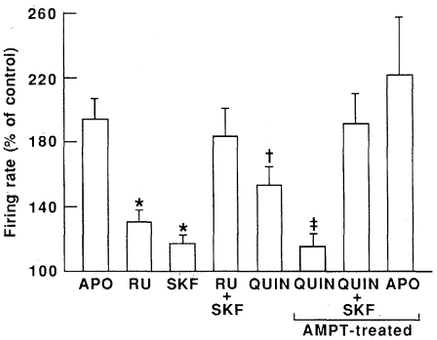

Fig. 2. Bar graph showing averaged effects of selective and nonselective D_1-D_2 dopamine agonists on globus pallidus neuron activity in normal animals and animals pretreated with AMPT (*23*). Drugs were administered intravenously at doses shown in Fig. 1, (A) through (F). Bars represent the mean and SEM. The number of neurons (that is, the number of animals) studied was: APO, 21; RU, 8; SKF, 43; RU + SKF, 12; QUIN, 12; QUIN + AMPT, 10; QUIN + SKF + AMPT, 9; APO + AMPT, 5. *, Significantly different (*27*) from apomorphine in normal and AMPT-treated rats and from the combination of RU 24926 and SKF 38393; †, significantly different from apomorphine in normal and AMPT-treated rats; ‡, significantly different from quinpirole in normal rats, from the quinpirole and SKF 38393 combination, and from apomorphine in normal and AMPT-treated rats.

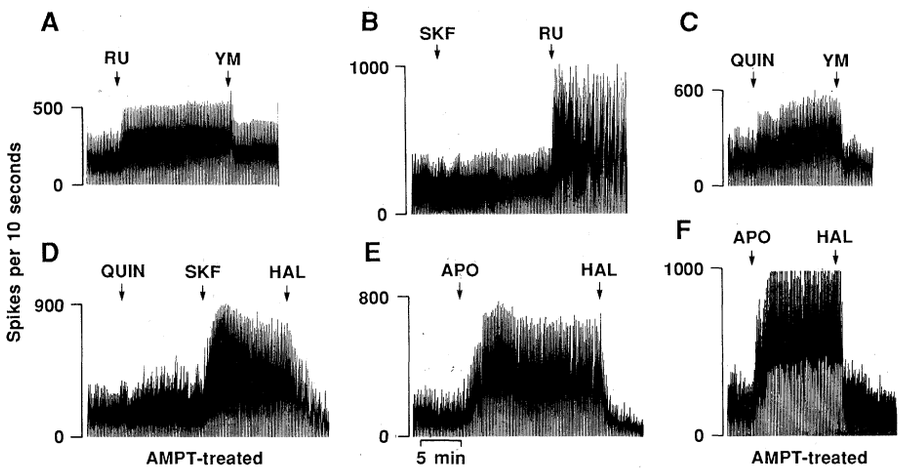

Fig. 1. Effects of dopamine agonists on the single unit activity of spontaneously active globus pallidus neurons. (**A**) RU 24926 (RU) (0.3 mg/kg), administered as a single bolus intravenous dose, induced modest increases in pallidal cell activity, which were reversed by administration of the D_2 antagonist, YM-09151-2 (YM) (0.2 mg/kg). (**B**) Administration of SKF 38393 (SKF) (20 mg/kg) had no significant effect on the firing rate of this pallidal neuron. However, when RU 24926 (0.3 mg/kg) was given 15 minutes after SKF 38393 (20 mg/kg), the resulting increase in firing was significantly greater than the effect observed when this dose of RU 24926 was administered alone. (**C**) Quinpirole (QUIN) (1 mg/kg) increased the firing rates of pallidal neurons. The dose of quinpirole administered here is greater than the doses of RU 24926 and apomorphine shown in (A) and (E), respectively, and is a maximally effective dose in the globus pallidus (*13*). YM-09151-2 (0.2 mg/kg) reversed the effect of quinpirole on firing rate. (**D**) In animals pretreated with AMPT (*23*), quinpirole (1.0 mg/kg) induced nonsignificant changes in pallidal cell activity. Subsequent administration of SKF 38393 (20 mg/kg) caused a rate increase similar to that induced by the combination of the D_1 and D_2 agonists shown in (B) and similar to that induced by the administration of apomorphine, a nonselective D_1-D_2 agonist, as shown in (E) and (F). This rate increase was reversed by haloperidol (HAL) (0.2 mg/kg). (**E**) Apomorphine (APO) (0.3 mg/kg) markedly increased pallidal neuron activity. (**F**) AMPT pretreatment (*23*) did not attenuate the effects of apomorphine (0.3 mg/kg).

logical effects (Table 1). In AMPT-treated rats, the ability of quinpirole to elicit the behaviors produced by this drug in normal animals was significantly diminished; frequencies of total motor activity as well as individual behaviors such as locomotion, sniffing, or grooming did not differ from those observed in controls receiving only vehicle and no AMPT. However, when quinpirole and SKF 38393 were administered in combination to AMPT-treated animals, marked stereotyped behavior resembling that typically seen after apomorphine or amphetamine administration was observed. Thus, a reduction in endogenous dopamine levels substantially attenuated the ability of quinpirole to induce behavioral changes in the AMPT-treated rat, while the full extent of apomorphine-like effects occurred only when the D_1 and D_2 receptors were stimulated simultaneously.

Our results indicate that D_2 dopamine receptors are not independently responsible for mediating postsynaptically induced effects of nonselective dopamine agonists such as apomorphine. Rather, a synergistic interaction between D_1 and D_2 dopamine receptor–regulated processes appears to be essential for the expression of behavioral phenomena typically associated with postsynaptically active doses of nonselective dopamine agonists, as well as the increases in tonic single unit activity in the globus pallidus evoked by systemic administration of these agents. Moreover, the behavioral or physiological effects induced by selective D_2 agonists apparently require the presence of endogenous dopamine concurrently stimulating the D_1 receptor. These conclusions are supported by a behavioral study that monitored dopamine agonist–induced rotation after a striatal quinolinic acid lesion (*6*) and by observations suggesting that the ratio of D_1 to D_2 receptor stimulation may determine the nature of behaviors expressed in intact and catecholamine-depleted animals (*24*).

Synergistic interactions between dopamine receptor subtypes also appear to occur in animals with supersensitive dopamine receptors; these interactions have been demonstrated by behavioral studies (*25*) and neurophysiological investigation of single unit activity in the substantia nigra pars reticulata, a second major basal ganglia output nucleus (*26*). Quinpirole and SKF 38393, at doses which, when given alone, induce no significant effect on substantia nigra pars reticulata neuronal activity will, when administered simultaneously, induce marked decreases in firing rates of these neurons (*26*). Thus, synergistic interactions between D_1 and D_2 receptors or between the processes they regulate appear to occur in the basal ganglia of both normal animals and animals with supersensitive dopamine receptors.

These results imply that, like the nonselective agonists, endogenous dopamine may induce certain of its behavioral and electrophysiological effects in the intact animal by concurrent stimulation of both receptor subtypes. In this respect, the central dopamine system seems unlike most central transmitter systems with multiple receptor subtypes studied thus far. Consideration of the functional interaction between D_1 and D_2 receptors could have important implications in disorders such as Parkinson's disease and schizophrenia, where stimulation or blockade of postsynaptic dopamine receptors confers symptomatic benefit. Knowledge of the optimal ratio of relative drug activity at D_1 and D_2 receptors may provide a basis for improved treatment in therapeutic situations where dopamine receptor stimulation or blockade is warranted.

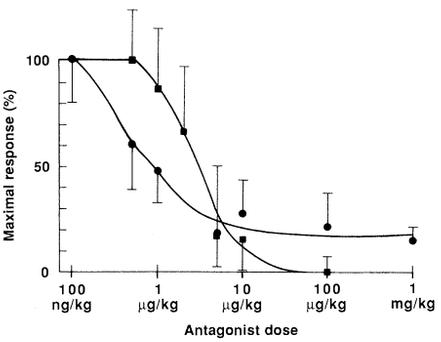

Fig. 3. Dose-response curves comparing the relative abilities of the D_1 antagonist SCH 23390 and the D_2 antagonist YM-09151-2 to block the excitatory effects of apomorphine on pallidal neuronal firing. SCH 23390 (●) or YM-09151-2 (■) were administered intravenously over a range of doses 3 minutes before administration of 0.3 mg/kg apomorphine. Only one dose was administered per experiment. Each point represents the mean response of all cells observed after apomorphine administration at a given dose of the antagonist (n = 5 to 10). Vertical bars represent SEM.

Table 1. Behavioral effects of the D_2 agonists quinpirole and RU 24926 and the D_1 agonist SKF 38393 administered independently and concurrently to control and AMPT-treated rats. Behavioral scores are expressed as the percent of the total number of intervals in which that behavior was observed. Male, Sprague-Dawley rats (250 to 300 g) were placed individually in 34 by 44 by 18 cm observation cages with 1 by 1 cm wire grid on the bottom and front walls and were allowed 2 hours to accommodate before testing. AMPT treatment was as described (*23*). Quinpirole, RU 24926, and SKF 38393 were administered subcutaneously in doses of 3 mg/kg, 3 mg/kg, and 20 mg/kg, respectively. SCH 23390 in a dose of 0.1 mg/kg was injected intraperitoneally. Behaviors were assessed by an observer unaware of the animal treatments. Specific behaviors (*28*) were rated as present or absent in a series of consecutive intervals uniformly distributed throughout the total period of observations. Observation intervals were generally 15 seconds in length and ranged from 10 to 20 in number. The total duration of behavioral observations ranged from 60 to 150 minutes in these experiments. Data were analyzed for statistical significance with Wilcoxon–Mann-Whitney Rank Sum tests and appropriate Bonferroni correction. The criterion of significance was $P < 0.05$.

Treatment	n	Nonstereotypic behaviors (% of time)			Intense stereotypy (% of time)	Total motor activity (% of time)
		Locomotion	Sniffing	Grooming		
Control	6	3 ± 2	5 ± 3	11 ± 3	0 ± 0	15 ± 4
Quinpirole	6	62 ± 11*†	62 ± 13*†	54 ± 13*†	0 ± 0†	100 ± 0*
SKF 38393	7	14 ± 5 †	32 ± 5 *†	52 ± 5*†	0 ± 0†	72 ± 6*
Quinpirole and SKF 38393	7	4 ± 2	4 ± 3	3 ± 2	86 ± 8*	100 ± 0*
RU 24926	6	26 ± 6*‡	68 ± 9*‡	45 ± 14*‡	0 ± 0‡	90 ± 5*
RU 24926 and SKF 38393	6	3 ± 2	9 ± 3	2 ± 2	82 ± 7*	99 ± 1*
Quinpirole	5	54 ± 16*‖	72 ± 14*‖	48 ± 13*‖	0 ± 0	100 ± 0*‖
Quinpirole and SCH 23390	6	1 ± 1§	9 ± 4§	5 ± 4§	0 ± 0	10 ± 4§
Quinpirole with AMPT pretreatment	6	11 ± 3§	6 ± 3§	4 ± 2§	0 ± 0	24 ± 4§
Quinpirole and SKF 38393 with AMPT pretreatment	8	4 ± 3§	2 ± 2§	1 ± 1§	95 ± 4*§‖	99 ± 1*‖

*Significantly different from control. †Significantly different from quinpirole and SKF 38393. ‡Significantly different from RU 24926 and SKF 38393. ‖Significantly different from quinpirole and SCH 23390 and also from quinpirole with AMPT pretreatment. §Significantly different from quinpirole.

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28. A behavioral checklist was utilized that allowed for independent quantification of individual motor behaviors as well as posture and movements of the head, mouth, trunk, and limbs. Behaviors such as locomotion or grooming were rated as present in an interval whenever these were documented on the checklist. Stereotyped behavior was rated independently. Stereotypy was rated as present in an interval when the animal received a score of 3 or greater on a modified Ernst Scale [H. L. Klawans, C. G. Goetz, P. M. Carvey, *Clin. Neuropharmacol.* **6**, 129 (1983)] or when characteristic repetitive and invariant patterns of head and limb movements were present.
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Proteolytic Self-Cleavage of Hepatitis B Virus Core Protein May Generate Serum e Antigen

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A model is proposed to explain the presence of the e antigen (HBeAg) of hepatitis B virus (HBV) in the serum of individuals infected with this virus. The e antigen, which has only recently been characterized, is a fragment of the virus core, or nucleocapsid, protein. Serum HBeAg is a valuable clinical marker for active HBV infection because its appearance correlates both with virus replication in the liver and with the presence of circulating virions. In this study a protease-like amino acid sequence was identified at the amino terminus of the core protein sequence. Experimental evidence indicates that HBeAg may be produced by proteolytic self-cleavage of the core protein.

THREE ANTIGEN COMPLEXES ARE ASsociated with infection by hepatitis B virus (HBV). The outer viral coat protein is detected as surface antigen (HBsAg) and the inner nucleocapsid protein as core antigen (HBcAg). The serum of infected individuals contains intact virions (Dane particles) and two viral antigens not associated with infectious particles: HBsAg and e antigen (HBeAg). Free HBsAg circulates as spherical and tubular particles: these noninfectious forms have been used to produce the serum-derived HBV vaccine. In contrast, HBeAg occurs as a soluble protein in the serum. The relationship of HBeAg to HBV has only recently been established. The finding of HBeAg reactivity in virus particles only when they were disrupted in vitro implied that HBeAg was present in the whole virus, but in a cryptic form. Further analysis demonstrated that both HBeAg and HBcAg reactivities were associated with the viral core protein and that HBeAg was, in fact, a fragment of the HBcAg molecule (*1, 2*). Analysis, by amino acid mapping, positioned HBeAg at the amino (NH_2) terminal half of the core molecule. The vast majority of soluble HBeAg in serum is not derived from HBcAg released from disrupted virions. It is, for an unknown reason, synthesized in excess during virus replication in hepatocytes and released from these cells as a soluble protein. Data presented here suggest that HBeAg may be produced by proteolytic self-cleavage of HBcAg in infected hepatocytes.

Human HBV is the prototypic member of the hepadnavirus family, which includes similar viruses which infect ground squirrels, woodchucks, and ducks. Recent studies show that the hepadnaviruses and retroviruses may be genetically related because they share a novel mechanism of genome replication involving reverse transcription of RNA (*3*), and because the nucleotide and predicted amino acid sequences of the genomes of these virus families share sequence homology (*4, 5*). Recent data also indicate that hepadnaviruses may express the polymerase protein as a nucleocapsid polymerase polyprotein, as do retroviruses (*6*). The protease gene sequence of retroviruses has been identified, and the putative active site has considerable homology with cellular acid, or carboxyl, proteases. However, no such protease gene sequence or protease activity has been identified in hepadnaviruses.

In this report I present evidence for a protease-like sequence in the predicted amino acid sequences of hepadnaviruses. The amino acid sequences of the hepadnavirus gene products (core, surface, polymerase, and X genes) were examined for sequences homologous with those of retroviral and cellular proteases. This analysis revealed the presence of a protease-like sequence located at the NH_2 terminus of the viral core protein sequence. This is likely to be a biologically

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