than the Swiss-Webster mouse to the neurotoxic effects of MPTP. Between seven and ten injections of MPTP in the C57 black mouse consistently led to about a 90 percent loss of neostriatal dopamine. These MPTP-treated mice exhibited postural abnormalities and had a marked reduction in their locomotor activity. In contrast, the Swiss-Webster mouse appears relatively normal even with losses of neostriatal dopamine greater than 80 percent.

In guinea pigs, 21 daily injections were required to induce a 50 percent decrease in dopamine concentrations (*4*). The differences in susceptibility to MPTP in different animal species are provocative and warrant further study. Differences in transport, absorption, metabolism, selective localization, or capacity to detoxify the neurotoxic species (either MPTP or some reactive metabolite or by-product) may be expected to account for these observations. Clarification of these differences may be important to an understanding of the mode of action of MPTP (*7*) and may yield clues to the etiology of Parkinson's disease in humans (*8*).

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7. In other experiments to define important structural aspects of MPTP, we gave 4-phenyl-1,2,5,6-tetrahydropyridine (PTP) and 1-methyl-1,2,5,6-tetrahydropyridine (MTP) to animals at a dose of 0.17 mmole per kilogram of body weight (equivalent to the 30-mg dose of MPTP). At this dose of each compound administered in injection sequences as described (see Table 1), we found no significant effects of PTP or MTP on dopamine concentrations. From these experiments we conclude that MPTP is considerably more potent than either PTP or MTP and that both the 1-methyl group and the 4-phenyl group are important in its effects.
8. Supported in part by grants from the U.S. Public Health Service and from the United Parkinson Foundation. We thank L. Manzino and F. S. Cabbat for assistance and W. J. Nicklas for helpful discussions.

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Identification of Common Interneurons Mediating Pre- and Postsynaptic Inhibition in the Cat Spinal Cord

Abstract. *The spike-triggered averaging of dorsal and ventral root potentials permits the identification of two populations of interneurons in the intermediate nucleus of the cat spinal cord. One produced negative dorsal root potentials and inhibitory ventral root potentials, in some cases with monosynaptic latencies, suggesting that they mediate presynaptic inhibition of group I afferent fibers from muscles and postsynaptic inhibition of motoneurons. The other population mediated only nonreciprocal postsynaptic inhibition of motoneurons.*

Primary afferent depolarization (PAD) is generally considered to cause presynaptic inhibition, but its nature and the mechanisms producing it remain uncertain (*1, 2*). This lack, together with the limited information available concerning the identity of the interneurons mediating the PAD, their connections, and the inputs received by them (*3–5*), has restricted our understanding of the functional role played by presynaptic inhibition in the vertebrate spinal cord.

Recent studies aimed at determining the sites of action of cutaneous and descending fibers on the pathways producing PAD of group I afferent fibers (*5*) have suggested that different last-order interneurons in the intermediate nucleus mediate the PAD of group Ia fibers (from muscle spindles) and Ib fibers (from tendon organs) (*5, 6*), and have provided criteria that, together with those already available (*1, 7, 8*), can be used for their characterization (*5*). Final identification of these interneurons, however, requires the demonstration of functional connections between them and their target afferent fibers, a condition not met in previous studies (*3, 4*). We have now used the spike-triggered averaging technique (*9–12*) to disclose the dorsal root potentials (DRP's) produced by single interneurons in the intermediate nucleus. These potentials are usually taken as indicators of synaptic actions exerted on afferent fibers (*1, 3, 6, 8*). Simultaneous spike-triggered averaging of ventral root potentials (VRP's) recorded with the sucrose gap technique (*9, 11*) has, in addition, allowed us to test the extent to which the PAD-producing interneurons form part of private pathways and determine whether they are also shared by other reflex pathways producing postsynaptic actions on motoneurons.

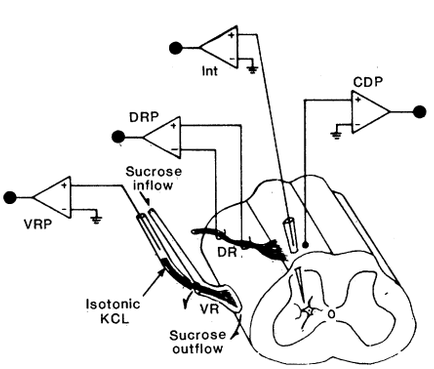

Fig. 1. Diagram of the method. Abbreviations: *Int*, interneuronal activity; *CDP*, cord dorsum potentials; *DR*, dorsal root; and *VR*, ventral root.

Cats were anesthetized with pentobarbital (35 mg per kilogram of body weight) supplemented (10 mg hourly) to maintain a deep level of anesthesia, paralyzed with Pavulon (pancuronium bromide), and connected to a respirator. The posterior biceps and semitendinosus, sural, gastrocnemius-soleus, deep and superficial peroneus nerves were dissected and prepared for stimulation. The lumbosacral spinal cord was exposed, and ventral roots L7 and S1 were dissected and sectioned distally. A small S1 dorsal rootlet was used to record the DRP's, and synaptic potentials of motoneurons were recorded from S1 ventral rootlets by means of the sucrose gap technique (*9, 11*) (Fig. 1). Action potentials of interneurons responding mono-, di-, or trisynaptically to group I gastrocnemius-soleus or posterior biceps and semitendinosus stimulation (*5, 6*) or polysynaptically to cutaneous nerve stimulation were extracellularly recorded by means of glass micropipettes (2- to 4-μm tip diameter) filled either with sodium chloride or with sodium glutamate (3*M*), the latter to increase the firing frequency of the neurons (*9, 10*). The DRP's and VRP's were recorded through low-noise differential amplifiers (band-pass filters, 0.1 Hz to 10 kHz). Cord dorsum potentials, DRP's, VRP's, and interneuronal activity were stored on analog tape. Averaging was performed on-line and off-line with a Nicolet 1170 computer.

We have analyzed the activation patterns and the connections with afferents and motoneurons of 130 interneurons located in the intermediate nucleus (laminae V and VI of Rexed). Eleven interneurons produced inhibitory VRP's (iVRP's) without any associated DRP's (Fig. 2, B and C) lasting 68.5 ± 25 msec (mean ± standard deviation). The

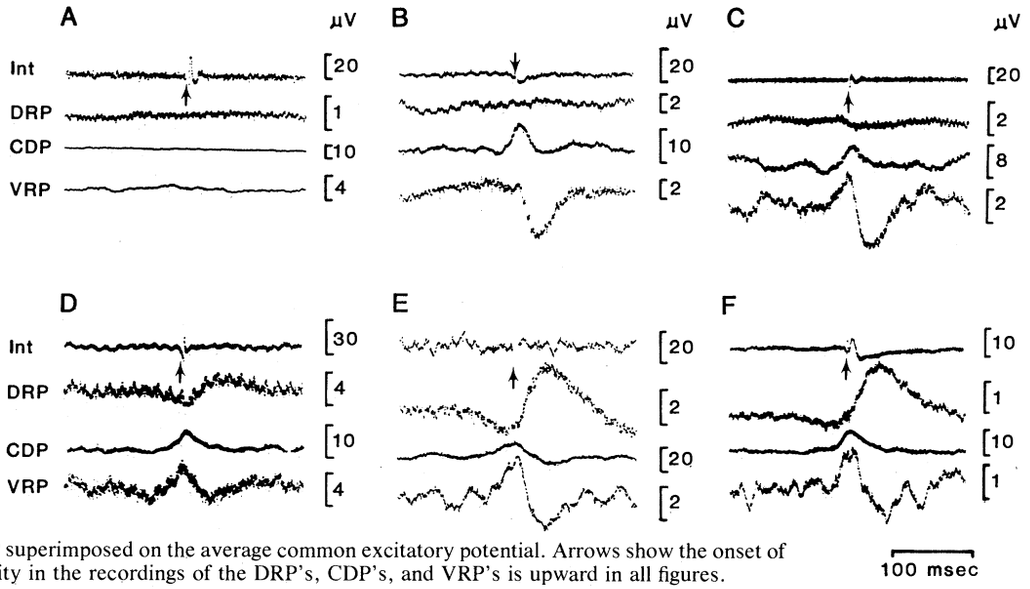

Fig. 2. Synaptic potentials generated in afferent fibers and in spinal motoneurons by single interneurons in the intermediate nucleus. This figure illustrates the main types of DRP's and VRP's generated by interneurons of laminae V and VI. (A) The interneuron failed to produce any DRP or VRP. (B and C) Interneurons produced inhibitory VRP's and no DRP's. Note the presence of the average common excitatory potential in the cord dorsum recordings in (B) and (C) and in the VRP recordings in (C). (D) The interneuron seems to produce a very small DRP without obvious inhibitory actions on motoneurons. (E and F) A large DRP after the interneuronal spike and an inhibitory component of the VRP superimposed on the average common excitatory potential. Arrows show the onset of the interneuronal spike. Negativity in the recordings of the DRP's, CDP's, and VRP's is upward in all figures.

iVRP's produced by six cells were preceded by a negative wave (Fig. 2C), the average common excitatory potential of Kirkwood and Sears (*13*) and of Brink *et al.* (*9*). The other five interneurons produced iVRP's that were not preceded by average common excitatory potentials (Fig. 2B). The latency of the iVRP's, measured from the onset of the interneuron spike, varied from 1.8 to 3.1 msec, the shortest values suggesting monosynaptic inhibitory connections with motoneurons (*9*).

Four of the iVRP-producing interneurons were activated monosynaptically by group I muscle volleys and polysynaptically by low-threshold cutaneous fibers [range, 1.12 to 1.25 times the threshold value ($\times T$)], and were fired antidromically from the Clarke's column nucleus in the rostral L4 segment. It thus seems that this group of cells (which we will term type A) mediates disynaptic nonreciprocal postsynaptic inhibition of motoneurons, in agreement with the recent observations of Jankowska and collaborators (*9, 14*), without being involved in the generation of the PAD (see also *15, 16*). The other seven interneurons (type B) produced iVRP's with latencies between 1.8 and 5.1 msec, and were activated polysynaptically by high-threshold muscle afferents (2.5 to 5 $\times$ T) and also by cutaneous nerves (1.11 to 2.28 $\times$ T). In one case, the interneuron was activated antidromically from Clarke's column.

Perhaps the most interesting observation is the finding of a substantial number of interneurons producing negative DRP's (nDRP's) as well as inhibitory VRP's (n = 9) (Fig. 2, E and F, and Fig. 3, C and D). The interneuronally evoked nDRP's and iVRP's had a time course resembling that of the DRP's produced by electrical stimulation of muscle nerves (Fig. 3, A, C, and D), with peak amplitudes of 2.4 ± 1.1 μV and of 2.0 ± 1.4 μV and durations of 132 ± 34 msec and 101 ± 23 msec, respectively. The nDRP's were 1/10 to 1/20 the size of DRP's produced by single pulses applied to the gastrocnemius-soleus and posterior biceps and semitendinosus nerves (39.2 ± 25 μV and 41.2 ± 28 μV, respectively). This may indicate a widespread action of a single interneuron on many afferent fibers, in agreement with previous suggestions (*17*).

The nDRP's produced by seven neurons started from a nearly flat or a slightly positive baseline, with onset latencies between 1.7 and 5.6 msec (Fig. 2E and Fig. 3, C and D). The other two interneurons produced nDRP's preceded by a small negative component, resembling the average common excitatory potentials recorded from the cord dorsum (Fig. 2F). The latencies of the nDRP's were 1.7 and 3.3 msec. The iVRP's produced by these interneurons were generally preceded by average common excitatory potentials (Fig. 2, E and F, and Fig. 3, C and D), but their estimated latencies were similar to those of the simultaneously recorded nDRP's (r = 0.75) and varied from 1.7 to 6.1 msec (Fig. 3D).

Three of the above neurons (type C) were activated mono- or disynaptically by group I fibers and polysynaptically by intermediate threshold (range 1.22 to 2.5 $\times$ T) cutaneous fibers; they could therefore mediate the postsynaptic inhibition of motoneurons produced by the group Ib afferents, and the flexor reflex afferents, as well as the PAD of group Ib fibers (*5, 7*). The interneuron of Fig. 3 was disynaptically activated by group I gastrocnemius-soleus volleys and produced nDRP's and iVRP's with monosynaptic latencies, as expected for a last-order interneuron in this pathway. Four other interneurons (type D) produced nDRP's and iVRP's with latencies from 1.7 to 4.3 and 1.7 to 5.6 msec, respectively, and were activated polysynaptically by cutaneous fibers (1.4 to 2.95 $\times$ T), but not by group I fibers in the gastrocnemius-soleus, posterior biceps and semitendinosus, and deep peroneus nerves. They could mediate the PAD of group Ib fibers (*5, 8, 9*), although it is not clear if they utilize the same pathways as type C interneurons.

Two neurons (type E) were activated exclusively by group I gastrocnemius-soleus fibers (latency, 1.2 and 1.8 msec), and produced nDRP's (latency, 2.3 and 3.7 msec), as well as iVRP's (latency, 2.9 and 4.3 msec). These neurons could be interposed in the pathway which mediates the PAD of group Ia fibers (*5, 6, 8, 9*). The remaining 108 neurons failed to produce any DRP's or VRP's (Fig. 2A), so their axonal connections remain undetermined.

The wide range in latencies of the interneuronally evoked potentials may reflect, to some extent, variations in the number of synapses interposed between the interneurons and their target elements (*6*). However, because of their variability, the contribution of polysynaptic components to the potentials is probably minimized with averaging techniques (*17*). It is therefore possible that the interneuronally evoked potentials are produced by activation of pathways with very few interposed synapses. In fact,

the shortest observed latencies of the potentials evoked by the same interneuron (1.7 to 1.87 msec) (Fig. 3, C and D) are compatible with monosynaptic delays, particularly if it is considered that they include the conduction time from the interneuron to the afferent fibers or motoneurons (0.2 to 0.3 msec) (*6, 9*), and the delay introduced by the electrotonic recording of synaptic potentials in the dorsal or ventral roots (0.2 msec) (*9*).

It thus appears that the same last-order interneurons in the pathway mediating the PAD group Ib fibers also produce postsynaptic inhibition of motoneurons. This explains the positive correlation observed between the latencies of the nDRP's and iVRP's produced by the same interneuron and implies in addition that both of them are produced by the same transmitter substance, presumably γ-aminobutyric acid (*1, 8, 18, 19*). This implication is consistent with the existence of picrotoxin-sensitive, strychnine-resistant inhibitory postsynaptic potentials elicited by group I fibers in spinal motoneurons with the same time course as the PAD (*20, 21*).

The proposal that a fraction of the intermediate nucleus interneurons activated by group I inputs is common to pathways mediating the PAD of group Ib fibers and postsynaptic inhibitory actions on motoneurons also implies that the Ib-mediated depression of group Ia excitatory postsynaptic potentials in spinal motoneurons cannot be attributed to pre- or to postsynaptic inhibition alone (*20, 22, 23*), but to both of them.

It is surprising that we could find only two interneurons that fulfilled the criteria expected for the interneurons mediating the PAD of group Ia fibers. The synaptic actions of these interneurons may be relatively weak and require temporal summation to be detectable (*6, 7*). Since the latencies of the nDRP's and iVRP's evoked by these interneurons were probably not monosynaptic, it is not possible to decide whether the last-order interneurons mediating the PAD of Ia fibers belong to private pathways, or if they are shared by pathways mediating postsynaptic inhibition of motoneurons. The observations of Fyffe and Light (*24*) of synaptic boutons of interneurons making axo-axonic synapses with identified Ia fibers in the motor nucleus and also with the motoneurons with which these fibers synapse support the second possibility, but additional information is necessary to define how frequent these arrangements are.

The presence of neuronal activity in advance of the interneuronal spikes used to evoke the averages (Fig. 2, C, E, and

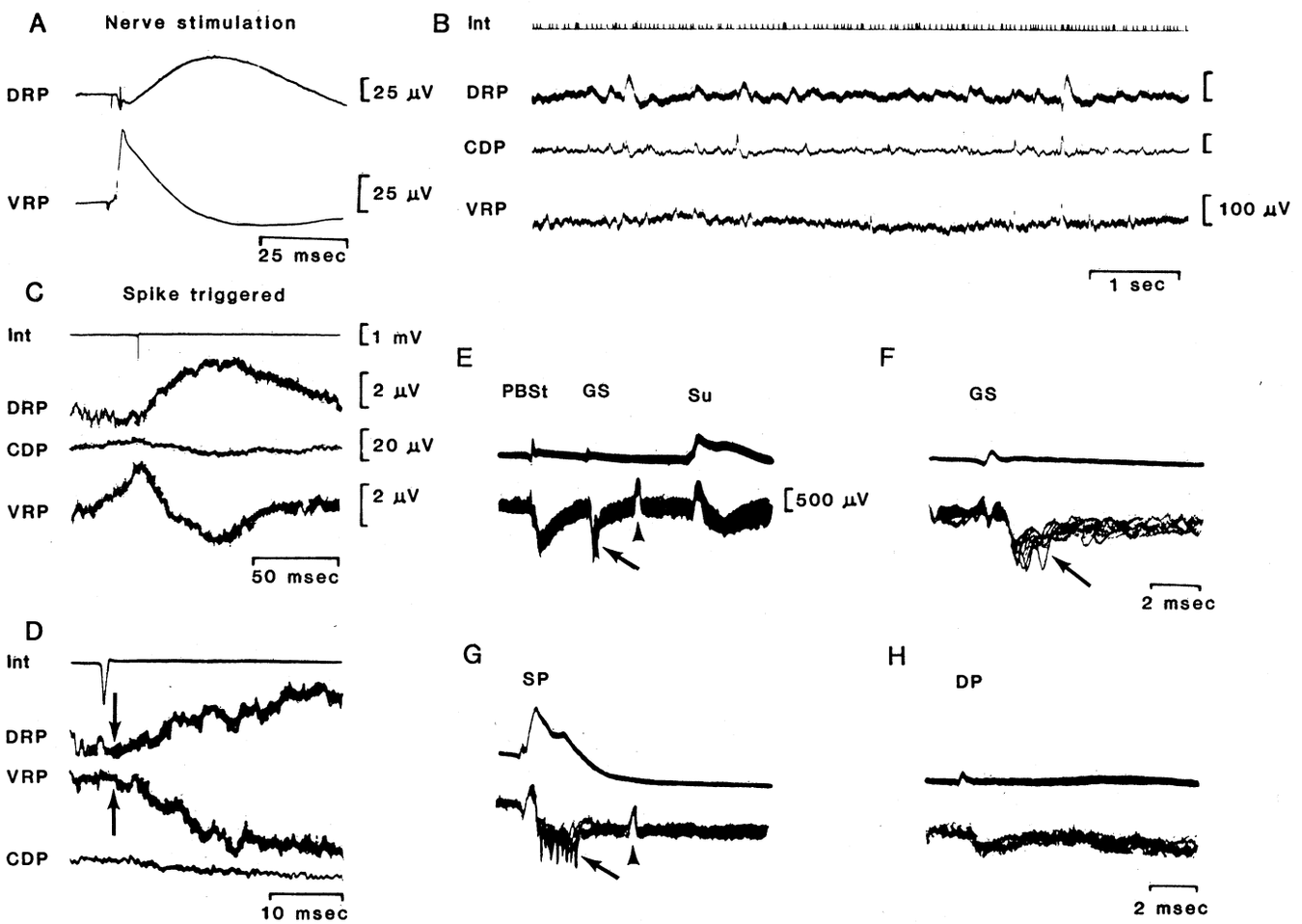

Fig. 3. Activation patterns of an interneuron producing nDRP's and iVRP's. (A) DRP and VRP produced by electrical stimulation of the gastrocnemius-soleus nerve with pulses 2 × *T* strength applied at 1 Hz. Each trace represents 64 averaged responses. (B) From above downward, the window discriminator output that gave a pulse whenever there was interneuronal firing, DRP's, CDP's, and VRP's recorded with a slow time base. (C and D) The spike-triggered averages of these potentials at two different time bases. Upper traces, averaged interneuronal spike. Arrows indicate approximate onset of the nDRP and iVRP, respectively. (E to H) Interneuron responses to stimulation of various nerves with 2 × *T* strength pulses. Upper traces, CDP's; lower traces, microelectric recordings. This interneuron responded disynaptically to stimulation of group I gastrocnemius-soleus (*GS*) afferents and polysynaptically to superficial peroneus (*SP*) afferents (arrows). The calibration pulses (arrowheads) in (E) and (G) are 500 μV and 1 msec.

F) raises the question of which spinal neurons are ultimately responsible for the observed nDRP's and iVRP's. Although our experimental method does not allow a direct answer, we believe that the finding of distinct groups of interneurons with specific and consistent activation and connectivity patterns supports the view that the investigated cells do mediate the actions attributed to them (*9*). The interneurons we have assumed mediate the PAD of group Ib fibers seem to be consistently activated by cutaneous volleys with strengths above $1.22 \times T$, which is about the minimal intensity required to evoke PAD of group Ib fibers (*5, 7*). The sets of neurons mediating nonreciprocal postsynaptic inhibition of motoneurons without producing PAD are instead activated by cutaneous fibers with strengths below $1.25 \times T$. In other words, our investigations document the existence of at least two distinct groups of inhibitory interneurons in the intermediate nucleus with different activation patterns and synaptic connections; these interneurons may play different roles in sensory discrimination and motor control.

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Sequencing the *erb*A Gene of Avian Erythroblastosis Virus Reveals a New Type of Oncogene

Abstract. *Avian erythroblastosis virus (AEV) contains two distinct oncogenes,* erb*A and* erb*B. The* erb*B oncogene, which is homologous to a portion of the epidermal growth factor receptor, is related to the* src *family of oncogenes and efficiently transforms erythroblasts, whereas* erb*A potentiates the effects of* erb*B by blocking the differentiation of erythroblasts at an immature stage. This "potentiator" was sequenced; the amino acid sequence deduced from it was clearly different from the sequences of other known oncogene products and was related to carbonic anhydrases. These enzymes participate in the transport of carbon dioxide by erythrocytes, the precursors of which are main targets of avian erythroblastosis virus. A* src*-related oncogene such as* erb*B in synergy with an activated specific cell-derived gene such as* erb*A can profoundly affect early erythroid differentiation.*

Two distinct oncogenes can act in synergy or complementation to transform normal cells (*1–3*). Some retroviruses contain two specific cell-derived sequences in their genome (*4–6*), probably leading to their selection as highly transforming viruses.

Avian erythroblastosis virus (AEV), a defective leukemia virus that transforms mainly erythroblasts (*7, 8*), contains two independent oncogenes, *erb*A and *erb*B (*9–14*), expressed in transformed cells from two distinct messenger RNA's (mRNA's) (*13, 15, 16*). A 5.4-kilobase (kb) mRNA codes for a cytoplasmic $P75^{gag\text{-}erbA}$ fusion protein (*17, 18*) while a subgenomic spliced 3.5-kb mRNA produces a 65 to 68-kilodalton (kD) membrane-associated glycoprotein (*19–21*). Both *erb*A and *erb*B oncogenes are ho-

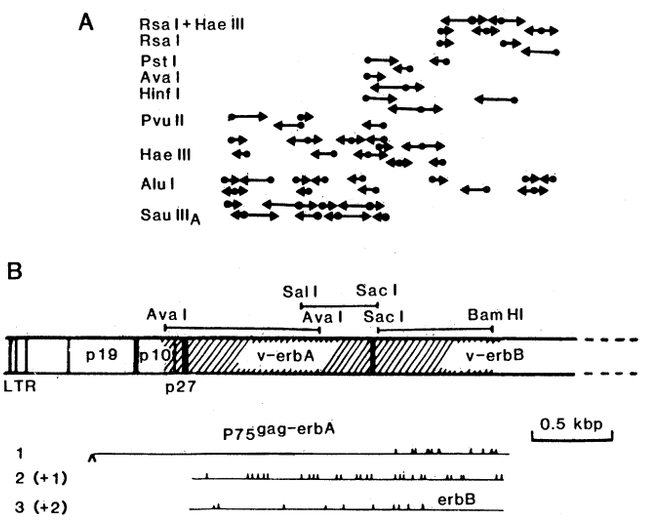

Fig. 1. (A) Sequencing strategy. The complete AEV DNA is represented linearized by Eco RI (*12*). The *erb*A sequence was obtained from three subclones, and solid bars indicate the *gag-erb* DNA inserts: 1-kbp Ava I–Ava I, 0.5-kbp Sal I–Sac I, and 0.7-kbp Sac I–Bam HI. Gel-purified restriction fragments used for DNA sequencing (restriction enzymes in the column at left) were labeled at their 5′ termini with [γ-^{32}P]adenosine triphosphate and polynucleotide kinase and sequenced by the method of Maxam and Gilbert (*31*). Closed circles indicate the cleavage points inside of each insert and horizontal arrows the direction and length of the sequenced DNA strands. (B) Reading frames in the genome of AEV. The DNA sequence was determined (hatched). The recombination of *erb*A with the viral genome occurred at the sites shown as thick vertical bars in the diagram of AEV DNA. p27 denotes the remaining coding domain for the p27 protein of the *gag* gene, which was truncated during the recombination events leading to capture of the *erb* insert. The open reading frame for $P75^{gag\text{-}erbA}$ and the two other reading frames in *erb*A are shown below the AEV DNA. A different open reading frame was detected for *erb*B. Vertical bars denote stop codons. LTR indicates a long terminal repeat.