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A LANT6-like substance that is distinct from neuromedin N is present in pallidal and striatal neurons in monkeys

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The basal ganglia of rhesus and squirrel monkeys were examined using immunohistochemical techniques with antibodies against the neurotensin-related hexapeptides Lys^8-Asn^9-Neurotensin(8–13) (LANT6) and Neuromedin N. A high percentage of neurons in both segments of globus pallidus and many large neurons of the striatum were found to label for LANT6, but not Neuromedin N. Previous studies have shown that LANT6 or a LANT6-like substance is present in many pallidal neurons in a wide range of vertebrate species. The current results indicate that a LANT6-like substance that is distinct from Neuromedin N is also present in many pallidal neurons in primates. This raises the possibility that this substance may be involved in neurotransmission between the pallidum and its projection targets.

The neurotensin-related hexapeptide Lys^8-Asn^9-Neurotensin(8–13) (LANT6)[5,6,7] has been found in the vast majority of pallidal neurons in the avian basal ganglia[28,29]. Many of these neurons have been shown to give rise to a LANT6-containing projection to tegmental dopaminergic neurons. Biochemical and immunohistochemical studies have also revealed the presence of a LANT6-like peptide in many pallidal neurons of the basal forebrain in turtles and hamsters[28,29]. Although the substance in pallidal neurons in turtles and hamsters may differ from avian LANT6 as sequenced by Carraway and Ferris[4], the LANT6-like peptide found in pallidal neurons in these animal groups is not neuromedin N, a neurotensin-related hexapeptide isolated originally from porcine spinal cord and differing from LANT6 by the substitution of isoleucine for asparagine as the second amino acid[19], or neurotensin itself. Immunohistochemical studies have shown that a LANT6-like peptide is also present in pallidal neurons in other reptiles (crocodilians), as well as in bony fish (lungfish) and cartilaginous fish (sharks)[28–30]. Since pallidal neurons are thought to use γ-aminobutyric acid (GABA) as their neurotransmitter[17], the above results suggest that LANT6 and GABA may co-occur in pallidal neurons. Double-label studies specifically examining this issue in pigeons have shown that GABA and LANT6 do co-occur in many pallidal neurons of the avian basal telencephalon[26]. Thus, the studies noted above raise the possibility that pallidal neurons in a wide variety of vertebrate species may use GABA, as well as LANT6 or a LANT6-like substance, to influence neurons in their projection targets. Although no data are available on the influence of LANT6 on neurons in the target areas of pallidal neurons, several lines of evidence suggest that LANT6 may influence these target neurons. First, LANT6 is highly concentrated in synaptosomal fractions of avian brain, suggesting that it may be a neuroactive substance released by synaptic terminals[6]. Second, LANT6 does appear to bind to neurotensin receptors, although with low affinity[9,16], and neurotensin receptors are rich in at least one target area of pallidal neurons, namely the dopaminergic neurons of the tegmentum[3,21,22,25]. Finally, substances related in structure to LANT6 (such as Neuromedin N and

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neurotensin) are known to activate tegmental dopaminergic neurons[1,13–15,33]. In the light of the possible role of LANT6 (or LANT6-like substances) as a neurotransmitter utilized by pallidal neurons and in the light of its phylogenetic ubiquity in pallidal neurons, the present studies were undertaken to determine if a LANT6-like peptide is also present in pallidal neurons in primates.

One rhesus monkey and two squirrel monkeys were deeply anesthetized with sodium pentobarbital and perfused transcardially with 0.1 M phosphate buffer (pH 7.2) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). The brains were removed, postfixed for 2–6 h and then stored in a solution of 20% sucrose–10% glycerin–0.1% sodium azide in 0.1 M phosphate buffer (pH 7.2) at 4 °C until

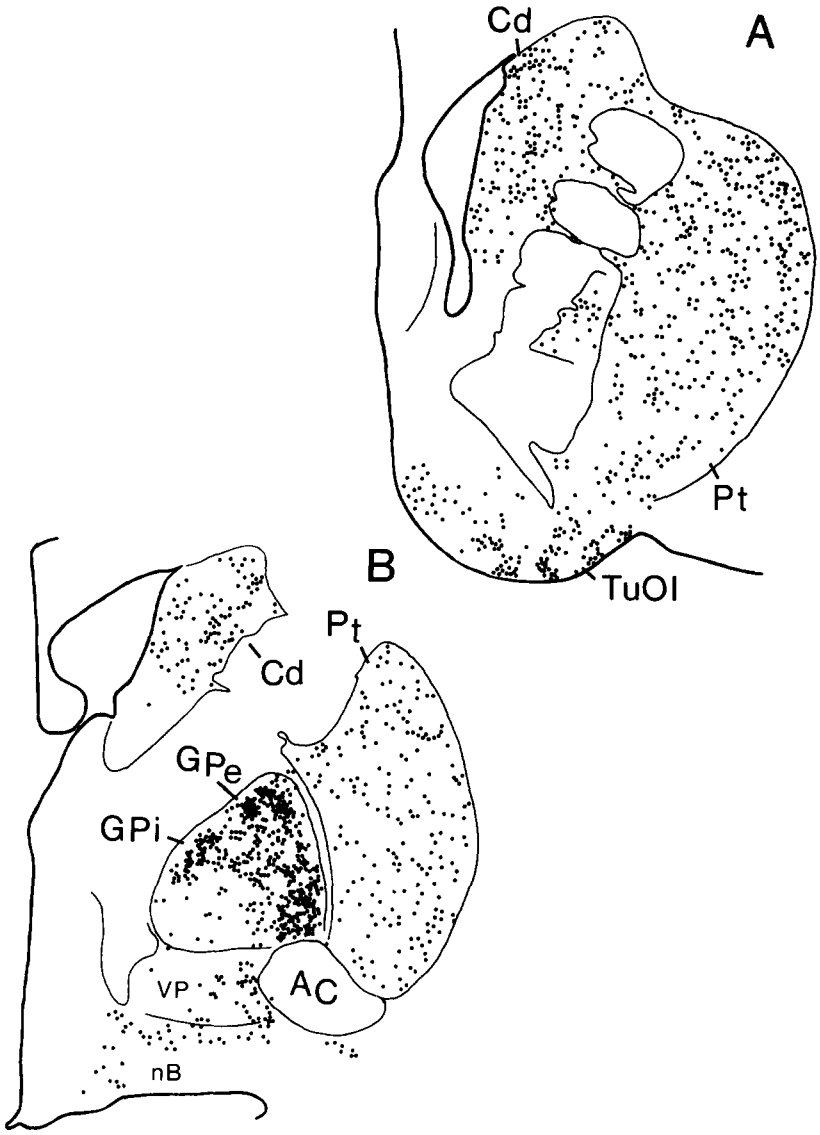

Fig. 1. Line drawings illustrating the distribution of LANT6+ neurons in the basal telencephalon of rhesus monkey at a rostral (A) and a caudal (B) level of the basal ganglia. Each dot represents one labeled cell body. The section thickness was 40 μm. The low numbers of labeled cell bodies in the ventromedial portion of GPi in part reflect the low density of LANT6+ neurons in this region, but also reflect the overall lower density of neurons in this portion of the pallidum. AC, anterior commissure; Cd, caudate; GPe, external segment of globus pallidus; GPi, internal segment of globus pallidus; nB, nucleus basalis/diagonal band of Broca region; Pt, putamen; TuOl, olfactory tubercle; VP, ventral pallidum.

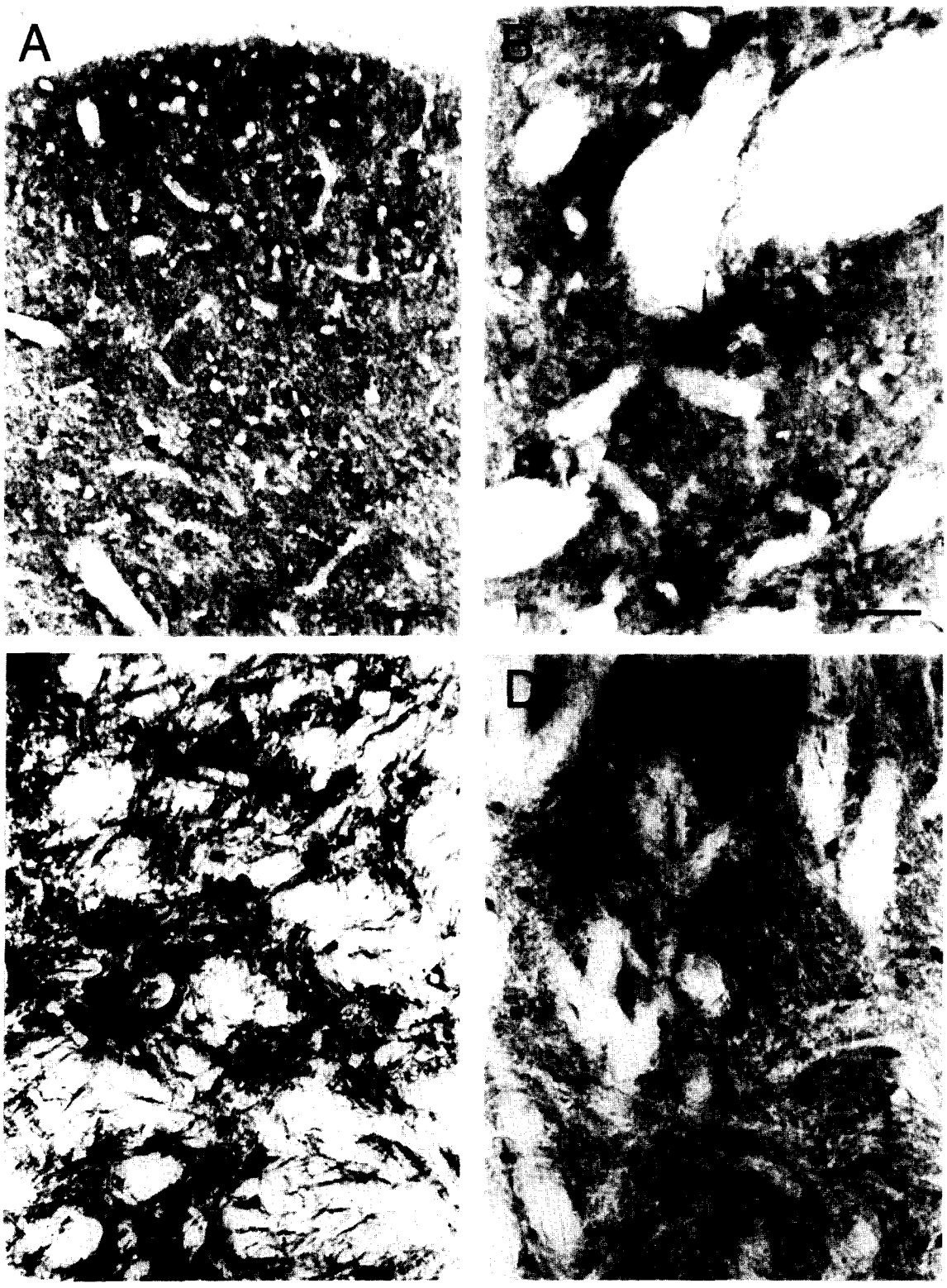

Fig. 2. Photomicrographs of LANT6+ neurons in the basal ganglia of monkeys. A: LANT6+ striatal neurons in rhesus monkey. B: a single LANT6+ striatal neuron at a slightly higher power in rhesus monkey, showing some dendritic labeling. C: LANT6+ pallidal neurons in the lateral pallidal segment of rhesus monkey. D: LANT6+ neurons in the medial pallidal segment of squirrel monkey. Bars: A = 100 μm; B = 250 μm. Bar in A applies also to C and D.

sectioned. Sections through the basal ganglia were sectioned at 30–40 μm on a sliding microtome and processed according to the peroxidase–antiperoxidase (PAP) procedure as described previously[28,29], using antisera against either neurotensin (purchased from the ImmunoNuclear Corporation), Neuromedin N (NMN) (provided by R.E. Carraway) or LANT6 (provided by R.E. Carraway). The specificity of these antisera for their target peptides has been confirmed in previous studies[6,28,29] and was also evaluated in the present studies. In brief, the anti-LANT6 antiserum is specific for the N-terminus of LANT6 and does not cross-react with either neurotensin or NMN. While neither the anti-neurotensin antiserum nor the anti-NMN antiserum cross-react with LANT6, they do cross-react slightly with one another's antigens.

Using the anti-LANT6 antiserum, abundant heavily labeled neurons were found in both segments of the globus pallidus (Figs. 1,2). Neuronal labeling was not observed in either segment of globus pallidus with either the anti-neurotensin antiserum or the anti-NMN antiserum. The perikarya of the LANT6+ neurons in globus pallidus were either ovoid or polygonal in shape, with the former ranging from 20 to 30 μm in diameter and the latter from 20 to 30 μm in the short axis and 30–40 μm in the long axis. Pallidal neurons in adjacent sections stained with Cresyl violet were typically of a similar size and shape as the LANT6+ pallidal neurons. In rhesus monkey, the number of LANT6+ neurons in globus pallidus per unit area in immunohistochemically processed sections was compared to the number of neurons in globus pallidus per unit area in adjacent Nissl-stained sections. The results indicated that a greater percentage of the neurons in the lateral globus pallidus (approximately 75%) were labeled for LANT6 than in the medial globus pallidus (approximately 50%).

LANT6+ neurons were also observed in the striatum (Figs. 1,2). These neurons were not labeled with either the anti-neurotensin antiserum or the anti-NMN antiserum. These LANT6+ striatal neurons were also large (approximately 20 μm) and their perikarya were typically round or polygonal in shape. These striatal neurons made up only a small percentage of the total number of striatal neurons (less than 1%). In Nissl-stained sections, neurons of this size and shape were also observed to be rare and make up less than 1% of the total number of striatal neurons (the vast majority of which, as reported by others, are medium-sized)[7]. Since the dendrites of these LANT6+ neurons were generally not well-labeled, the dendritic morphology of these neurons could not be well characterized. In the case of those perikarya where some dendritic labeling was observed, 3 or more thin dendrites were observed to extend from the cell body. The LANT6+ neurons were found throughout the striatum and, as shown in Fig. 1, they were not uniformly distributed in the striatum. To determine the relationship of the distribution of these neurons to the cholinesterase-poor patches of the striatum[10], the distribution of LANT6+ neurons was compared to the cholinesterase staining pattern (using the procedure of Mesulam and Van Hoesen[18]) in adjacent sections. The results indicated that, although LANT6+ neurons are not uniformly distributed in the striatum, neither regions of high LANT6+ perikaryal density nor regions of low LANT6+ perikaryal density consistently correlated with either ACHE-poor or ACHE-rich zones. Thus, LANT6+ neurons are present in both patch and matrix compartments of the striatum. Finally, as in pigeons, turtles and hamsters[28,29], LANT6+ neurons were also observed in the ventral pallidum and in the polymorph layer of the olfactory tubercle (Fig. 1). Additional LANT6+ neurons were observed in the diagonal band of Broca/nucleus basalis portion of the telencephalon. As in the case of the striatum and globus pallidus, the LANT6+ neurons in these regions were large (20–30 μm).

The present results thus show that in primates, as in hamsters and a variety of non-mammalian species[28–30], a LANT6-like substance is present in neurons of globus pallidus. Although the precise identity of the LANT6-like substance in pallidal neurons in monkeys is unknown, this substance clearly is distinct from NMN and neurotensin (which are related in structure to LANT6), since pallidal neurons were not labeled with antisera against these substances. These latter antisera did, however, label neurons and fibers in other brain regions, thus suggesting that their failure to label pallidal neurons reflects the absence of neurotensin and NMN from pallidal neurons. Thus, as also recently shown in another mammalian species (i.e. hamsters)[29], the present results show that the nervous system of monkeys contains neurotensin,

NMN and a distinct LANT6-like peptide, and that it is the LANT6-like peptide that is present in pallidal neurons. Previous studies in birds have shown that LANT6 is present in GABAergic pallidal neurons[26]. Pallidal neurons in mammals are also thought to use GABA as their transmitter. In the light of this and in the light of the large number of LANT6+ pallidal neurons observed in monkeys, it appears likely that GABAergic pallidal neurons in monkeys contain a LANT6-like peptide.

The present results show that LANT6+ neurons are also present in the striatum in monkeys. These neurons are large, presumably aspiny, neurons that make up fewer than 1% of the total number of striatal neurons. These neurons are present in both patch and matrix compartments. LANT6+ striatal neurons are also present in the basal ganglia of birds and reptiles, but have not been observed in hamsters[29]. The failure to observe LANT6+ striatal neurons in hamsters is of uncertain significance. Possibly these neurons are absent in hamsters, or possibly the LANT6-like substance they putatively contain differs considerably from avian LANT6 and is only poorly detected by the present antiserum against LANT6. Regardless, the present results clearly show that LANT6+ striatal neurons are demonstrable in at least some mammalian species. The precise relationship of these neurons to previously identified types of striatal neurons in mammals is uncertain. Since all LANT6+ striatal neurons in birds also contain GABA[26], it is possible that the LANT6+ striatal neurons in monkeys correspond to a class of large neurons that also contain GABA, such as the class of large striatonigral projection neurons reported in the striatum of some mammals[2]. The LANT6+ striatal neurons, however, also show morphological resemblances to cholinergic striatal neurons[24], and the possibility cannot be excluded that some of the LANT6+ striatal neurons contain acetylcholine. It is clear, however, that the LANT6+ striatal neurons differ in size and morphology from the neurotensin-containing striatal neurons recently described in cats by Sugimoto and Mizuno[31], which are medium-sized spiny neurons.

The precise functional significance of the presence of a LANT6-like peptide in pallidal neurons has not yet been determined. Existing data, however, raise the possibility that at least some pallidal neurons use LANT6 as a neurotransmitter in feedback regulation of the dopaminergic neurons projecting to the basal ganglia. First, in both birds and reptiles, many LANT6+ pallidal neurons have been shown to project to the tegmental dopaminergic cell groups[29]. Similarly, in mammals, the olfactory tubercle, the ventral pallidum and the lateral globus pallidus project to the ventral tegmental area and substantia nigra, which are rich in dopaminergic neurons[11,12,20,23,32], and it appears likely that LANT6+ neurons are among those projecting to these tegmental sites. Secondly, LANT6 has some affinity for neurotensin receptors and neurotensin receptors are present on tegmental dopaminergic neurons[9,16,22,25]. Finally, neurotensin and NMN (both of which are structurally related to LANT6) are known to activate dopaminergic neurons, thus suggesting that LANT6 may also be able to influence dopaminergic neurons[1,13–15,21,33].

LANT6+ neurons were also observed in the medial pallidum segment (which projects to the ventral anterior/ventral lateral nuclei and the centromedianum/parafascicular complex of the thalamus)[7,8,11,12,20,23]. Thus, it seems possible that medial pallidal neurons also use a LANT6-like peptide to influence their thalamic target neurons. However, since in rhesus monkey, as in hamsters, LANT6+ neurons are more abundant in the lateral portion of the pallidum than in the medial, the LANT6-like peptide in pallidal neurons in mammals may play a greater role in neurotransmission in the target areas of the lateral pallidum (which also include the subthalamic nucleus)[7,8,12,20], than in target areas of the medial pallidum. Further understanding of the role of LANT6 would be aided by clarification of the identity and distribution of the receptor sites to which LANT6 binds.

In summary, although much remains to be determined about the functional significance of the presence of LANT6 in pallidal neurons and of its apparent co-localization with GABA, it is clear that a LANT6-like peptide is present in pallidal neurons in vertebrate species as distantly related as sharks and primates. These results reinforce previous observations on the high degree of evolutionary conservatism in the neurotransmitter/neuropeptide-specific populations of neurons making up the basal ganglia[27]. Further, in the light of the striking evolutionary conservatism in the presence of LANT6 (or related substances) in pallidal neurons, it seems likely

that LANT6 plays a fundamental role in the functions of pallidal neurons.

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