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Origin of the Cholecystokinin-Containing Fibers in the Rat Caudatoputamen

Abstract. *Large amounts of cholecystokinin-octapeptide (CCK) are present in the rat caudatoputamen. The peptide occurs in axons and nerve endings but not in perikarya. The origin of CCK in the caudatoputamen was investigated with the use of immunocytochemistry and a radioimmunoassay specific for CCK. Although a small amount of CCK (approximately 30 percent) originates in the amygdaloid complex, the bulk of the peptide (approximately 70 percent) occurs in processes of neurons located ventral to the caudatoputamen, that is, the claustrum or the piriform cortex. The claustrum and piriform cortex receive inputs from various cortical areas and the olfactory system, respectively, and may process information and relay it to the caudatoputamen. Thus CCK may be the transmitter in the final common pathway linking various cortical areas and the olfactory system to the caudatoputamen.*

Several neurotransmitters have been found in the caudate nucleus (*1*). Some of these are made by neurons located within the nucleus itself (for example, acetylcholine, γ-aminobutyric acid, substance P, and enkephalin); others are made in processes of neurons that are found elsewhere in the brain (for example, dopamine and serotonin). Numerous studies of the interactions among neurons that innervate and constitute the caudate nucleus have contributed to a better understanding of its role in somatic motor function and the pathophysiology of Parkinson's and Huntington's diseases (*2*).

Cholecystokinin (CCK), a peptide hormone that stimulates pancreatic enzyme secretion and contracts the gall bladder, has been found in high concentrations in the caudatoputamen (*3*). Binding sites for this hormone have also been found there and appear to be on cells that are intrinsic to the nucleus (*4*). No CCK-positive neuronal perikarya have been found in the rat caudatoputamen (*5*), but a somewhat dense plexus of axons and nerve endings has been visualized. Thus it seemed that another structure must provide the caudate with CCK.

The caudate is innervated by the amygdala, ventral tegmental area, substantia nigra, dorsal raphe nucleus, thalamus, and several cortical areas (*6*). Except for the thalamus all of these areas have CCK-containing perikarya (*5*) and could potentially provide the caudate with CCK. We therefore examined the effect on the CCK content of lesions that selectively destroy caudate afferents. In addition, we used immunocytochemistry to visualize CCK-containing projections to the nucleus.

Unilateral brain lesions were made in female Osborne-Mendel rats anesthetized with ether. These lesions removed inputs to the caudate nucleus from the substantia nigra, amygdaloid complex, dorsal cerebral cortex, insular cortex, and the claustrum-piriform cortex. Seven days later the rats were killed by decapitation. The brains were quickly removed and coronal sections 2 mm in thickness were made with razor blades and a template. The part of the caudatoputamen approximately 7 mm rostral to the interauricular line was dissected from the sections and CCK was extracted and measured by radioimmunoassay (*3*).

A surgical lesion of the nigrostriatal pathway (*7*) caused the dopamine to disappear from the caudatoputamen (*8*) but did not decrease the total CCK-concentration (Table 1). This indicates that neurons in the substantia nigra contribute little CCK to the caudatoputamen (*9*). Similarly, neither separating the insular cortex (*10*) from the caudate nor removing those parts of the frontal, parietal, and occipital cortex that are accessible after a dorsal craniotomy (*11*) had any effect on the CCK concentration in the caudatoputamen (Table 1).

In contrast, a knife cut severing afferents from the amygdala reduced the CCK concentration in the caudatoputamen by approximately 30 percent (Table

Table 1. The effect of several knife cuts on the concentration of CCK in the head of caudate nucleus. The concentration of CCK (mean ± standard error) is given in nanograms per milligram of protein. The number of animals in each group appears in parentheses. Protein was measured according to Lowry *et al.* (*16*). The lesioned side was compared to the unlesioned side of the same animals (paired Student's *t*-test); N.S., not significant.

Area severed	Lesioned side	Control side	*P*
Substantia nigra	11.28 ± 2.22 (8)	6.34 ± 0.94 (8)	N.S.
Amygdaloid complex	5.55 ± 0.94 (11)	7.77 ± 0.79 (11)	.05
Dorsal cortex	9.1 ± 1.0 (5)	7.1 ± 1.0 (5)	N.S.
Insular cortex	4.98 ± 0.52 (8)	6.49 ± 0.57 (8)	N.S.
Claustrum-piriform cortex	2.63 ± 0.79 (11)	7.38 ± 1.61 (11)	.005

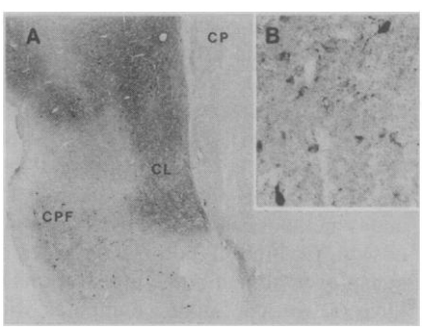

Fig. 1. Brain sections showing the distribution of CCK. Immunocytochemistry was performed according to Sternberger (*17*). Twenty-four hours after the injection of 25 μg of colchicine into the caudatoputamen (in 2 μl of vehicle; 9.2 mm anterior to the interauricular line, 2.5 mm lateral to the midline; 6.2 mm ventral to the dural surface, with the plane angle 5° down) rats were fixed with 4 percent paraformaldehyde in 0.1*M* sodium phosphate buffer (*p*H 7.35). The brains were removed and placed in fixative for 12 hours. After immersion in 30 percent sucrose for 48 hours 20- to 25-μm sections were cut on a cryostat or on a Vibratome. The sections were incubated with the immunoglobulin G fraction of an antiserum to CCK8-sulfate, conjugated to bovine serum albumin by glutaraldehyde which had been preabsorbed with liver acetone powder (dilution 1:300; incubation time 24 hours at room temperature). For staining, 3,3'-diaminobenzidine (5 mg/ml) was used in the presence of 0.01 percent hydrogen peroxide. In preabsorption control experiments antiserum (1:300) was first incubated with 3.75 μg of CCK8 per milliliter for 24 hours. This procedure abolished all staining. (A) A section showing the piriform cortex (*CPF*), claustrum (*CL*), and caudatoputamen (*CP*) (×38). (B) A section showing the claustrum with the perikarya staining for CCK (×220).

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1) (*12*). Thus, cells in the amygdaloid complex seem to provide the caudate with some of its CCK, but which amygdaloid cells do so remains to be determined.

In cats the caudate nucleus has been reported to receive projections from the claustrum, although the claustrum sends rather few of its axons there (*13*). Immunocytochemical studies of this structure and of the adjacent piriform cortex revealed heavy staining for CCK in both areas (Fig. 1A). In the claustrum, CCK was principally in axons, but scattered cell bodies were also stained (Fig. 1B). In the piriform cortex CCK was found mainly in cell bodies.

A knife cut severing the claustrum and the piriform cortex from the caudatoputamen (*14*) decreased the CCK concentration in the caudatoputamen by approximately 70 percent (Table 1). This knife cut did not compromise the blood supply to the caudate and caused a minimal amount of nonspecific, mechanical damage. The perikarya that stain for CCK in the piriform cortex are situated adjacent to the claustrum. Selective ablation of the piriform cortex was therefore impossible. Consequently, we could not determine whether the caudatoputamen receives its CCK-containing afferents from the claustrum, the piriform cortex, or both.

The claustrum receives afferents from various cortical areas whereas the piriform cortex is part of the olfactory system (*15*). Thus CCK might be the transmitter in the final common pathway linking various cortical areas or the olfactory system to the caudatoputamen. This finding suggests possible new directions for research on the basal ganglia and raises the possibility that CCK-containing neurons in the claustrum-piriform cortex, which provide most of the CCK found in the caudatoputamen, may be involved in regulating the caudatoputamen.

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7. A 2-mm blade was inserted 4.3 mm anterior to the interauricular line down to the bone, with the medial edge of the blade in the midline (plane angle 11° down). The rats weighed 200 to 250 g.
8. Dopamine concentrations were determined as described by R. M. McCarty, G. Gilad, V. K. Weise, and I. J. Kopin [*Life Sci.* **25**, 747 (1979)]. The dopamine concentration was 0.87 ± 0.07 μg per gram, wet weight (mean ± standard error; $N = 8$) in the unlesioned side and not measurable in the lesioned side.
9. The dopamine and CCK content of the nucleus accumbens was also measured in rats with unilateral lesions in the median forebrain bundle. The dopamine content was reduced 90 percent by this lesion whereas the CCK content was unchanged indicating that the mesencephalic regions such as the ventral tegmental area are not the source of the bulk of the CCK in the nucleus accumbens. See T. Hokfelt, L. Skirbol, J. F. Rehfeld, M. Goldstein, K. Marhey, and O. Dann [*Neuroscience* **5**, 2093 (1980)] for a description of the codistribution of dopamine and CCK.
10. Separation was achieved with a 5-mm blade inserted 11.0 mm anterior to the interauricular line down to bone; the angle of the blade to midline was 45°, the middle edge of the blade was 4.0 mm lateral to the midline, and the plane angle was 11° down.
11. After removing a bone flap covering the dorsal part of the brain, the accessible cortical areas were removed by suction.
12. The cut was made with a 3-mm blade, 7.5 mm anterior to the interauricular line down to bone, with the middle edge of the blade 2 mm lateral to the midline, and the plane angle 11° down.
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Substance P Analog, DiMe-C7: Evidence for Stability in Rat Brain and Prolonged Central Actions

Abstract. *A metabolically protected analog of substance P, [pGlu^5-MePhe^8-MeGly^9]SP(5–11) (DiMe-C7), was approximately equipotent with substance P in causing increased locomotor activity after microinfusion into the ventral tegmental area of rat brain, but the effects of DiMe-C7 on behavior were considerably prolonged. There was little metabolic degradation of tritiated DiMe-C7 for up to 1 hour after infusion, whereas tritiated substance P was completely degraded within 10 minutes.*

Substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) (SP) occurs in neurons in many parts of the mammalian central nervous system and is widely regarded as a putative neurotransmitter (*1–3*). Assessing the central actions of SP, however, is made difficult by the metabolic instability of this peptide in brain (*4*). We have recently synthesized an analog of SP ([pGlu^5-MePhe^8-MeGly^9]SP(5–11) (DiMe-C7) that is resistant to attack by a substance P–degrading enzyme purified from brain (*5*) and is metabolically stable when incubated with rat brain hypothalamic slices or synaptosomal membrane preparations in vitro (*5, 6*). The analog competes with ^{3}H-labeled SP for binding to receptor sites in rat brain membranes and also retains biological activity in various peripheral test systems, although its potency ranges from 1/10 to 1/50 of that of SP (*6, 7*).

We now present evidence that DiMe-C7 is metabolically stable when administered directly to rat brain and that it produces persistent behavioral responses. To assess the central actions of the analog we used a behavioral model previously developed in this laboratory, in which substance P or related compounds are microinfused in the region of dopamine cell groups in rat brain (*8, 9*). When SP is locally administered into the ventral tegmental area (VTA) or substantia nigra it causes characteristic patterns of hyperactivity (*8, 9*). Accordingly, we infused SP and DiMe-C7 into the rat VTA and determined the time course of the behavioral response. In parallel experiments with radioactive peptides (*10*) we examined the time course of disappearance of unchanged peptides after they were locally microinfused into the VTA.

Peptides were administered through stereotaxically implanted guide cannulas aimed at the VTA, and locomotor responses were recorded in photocell ac-

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