Nevertheless, a central lesion could be of pivotal importance in the development of the syndrome. To evaluate this, we analyzed brain catecholamines in the mice. Catecholamines have been implicated in abnormal endocrine function in *ob/ob* mice (*8*) and probably are important in hypothalamic control of feeding patterns (*9*). Catecholamine levels were determined in the brainstem, hypothalamus, and forebrain of five obese animals, five infected lean controls, and five uninfected lean controls by the analytical procedure of Hegstrand and Eichelman (*10*), which involves high-pressure liquid chromatography with electrochemical detection. Concentrations of dopamine and norepinephrine in forebrain specimens from obese animals were two to three times lower than in specimens from uninfected lean controls. Forebrain from infected lean controls had concentrations of dopamine and norepinephrine that were intermediate between those measured in forebrain from obese and uninfected controls. Catecholamine levels in hypothalamic specimens from infected animals, both lean and obese, were difficult to evaluate because of considerable variance. There were no significant differences in brainstem dopamine and norepinephrine between obese mice, infected lean controls, and uninfected lean controls. Thus a primary pathological process involving virus-induced destruction (or dysfunction) of select groups of neurons, resulting in defective catecholamine synthesis or release, may be critical in the development of the syndrome. Studies of a variety of other physiological and biochemical variables are needed to fully describe this type of obesity.

There is a clear role for virus infection in the initiation of a number of chronic disorders in man (*11*). The present findings raise the possibility that virus infection also plays a role in some cases of obesity in children and in some cases of severe spontaneous obesity in adults.

MICHAEL J. LYONS
IRVING M. FAUST
RICHARD B. HEMMES
DANIEL R. BUSKIRK
JULES HIRSCH
JOHN B. ZABRISKIE
Rockefeller University, New York 10021

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Dynorphin and Vasopressin: Common Localization in Magnocellular Neurons

Abstract. *The opioid peptide dynorphin is widely distributed in neuronal tissue of rats. By immunocytochemical methods, it was shown previously that dynorphin-like immunoreactivity is present in the posterior pituitary and the cells of the hypothalamic neurosecretory magnocellular nuclei which also are responsible for the synthesis of oxytocin, vasopressin, and their neurophysins. By using an affinity-purified antiserum to the non-enkephalin part of the dynorphin molecule it has now been demonstrated that dynorphin and vasopressin occur in the same hypothalamic cells of rats, whereas dynorphin and oxytocin occur in separate cells. Homozygous Brattleboro rats (deficient in vasopressin) have magnocellular neurons that contain dynorphin separate from oxytocin. Thus dynorphin and vasopressin, although they occur in the same cells, appear to be under separate genetic control and presumably arise from different precursors.*

The opioid peptide dynorphin, which was originally extracted from porcine pituitary, is highly active in the guinea pig ileum bioassay for opiate activity (700 times more potent than leucine-enkephalin) (*1*). By radioimmunoassay dynorphin has been demonstrated in the neurointermediate lobe of the pituitary, hypothalamus, cortex, hippocampus, medulla-pons, spinal cord, and dorsal-root ganglia of rats (*2, 3*). We previously demonstrated that immunoreactive dynorphin occurs in cells other than β-endorphin–producing cells and pituitary in rat brain (*4*). We located dynorphin in the posterior pituitary and the magnocellular nuclei of the hypothalamus (supraoptic and paraventricular nuclei). Since the magnocellular components of these nuclei are associated with the production of oxytocin, vasopressin, and the enkephalins (*5*), we undertook an anatomical study of the cellular distribution of dynorphin and these peptides. We now report the presence of immunoreactive dynorphin in cells that produce vasopressin. We also show that Brattleboro rats, known to be deficient in the production of vasopressin and its neurophysin (*6*), contain dynorphin in the non-oxytocin–containing cells of the magnocellular nuclei. This, along with a 300-fold difference in content of arginine vasopressin (AVP) and dynorphin (*2, 7*) suggests that the peptides are under different biosynthetic control.

Brain tissue was prepared from Sprague-Dawley rats for immunocytochemical study as reported elsewhere (*4*). In brief, normal rats and rats treated with colchicine (50 μg/50 μl, injected into the cerebral ventricles 48 hours before the rats were killed) were anesthetized with pentobarbital (50 mg/kg) and perfused by way of the aorta with 4 percent cold formaldehyde phosphate buffer (0.1*M*) at 4°C for 30 minutes. The brains were removed, blocked, and post-fixed in the same fixative and maintained overnight at 4°C in 0.1*M* phosphate-buffered normal saline, *p*H 7.2, with 15 percent sucrose. They were then frozen in liquid nitrogen and sectioned at −20°C on a cryostat. Serial 5-μm sections were cut through the hypothalamic magnocellular nuclei.

Immunocytochemical staining was carried out according to the PAP technique (*8*) with antiserums to dynorphin-(1–13), AVP, and oxytocin. Dynorphin-(1–13) was conjugated via glutaraldehyde to thyroglobulin as reported (*2*). To reduce nonspecific background reactivity in the immunocytochemical study we used affinity-purified and fully recharacterized dynorphin antibodies. The affinity resin used for antibody purification was prepared by linking dynorphin(1–13) to cyanogen bromide–activated Sepharose 4B with cyanogen bromide (*9*). The resulting antibodies were used at a 1/300 dilution; they were found to be blocked

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by 1 μ*M* dynorphin(1–13) (Fig. 1, A and B) but not by 50 μ*M* leucine-enkephalin, methionine-enkephalin, β-endorphin, AVP, oxytocin, or methionine-Arg^6, Arg^7-enkephalin. We therefore concluded that the affinity-purified antibodies bound to some part of dynorphin(6–13). Since natural dynorphin is a 17-amino acid opioid (*9*), the affinity-purified antibody can be construed as a "midportion" dynorphin antiserum. It is fully cross-reactive with the recently isolated hepta decapeptide from porcine pituitary (*9*). Furthermore, it readily detects dynorphin precursors from porcine pituitary and rat dynorphin from hypothalamus in tissue extracts or when partially purified by molecular sieving and high-pressure liquid chromatography.

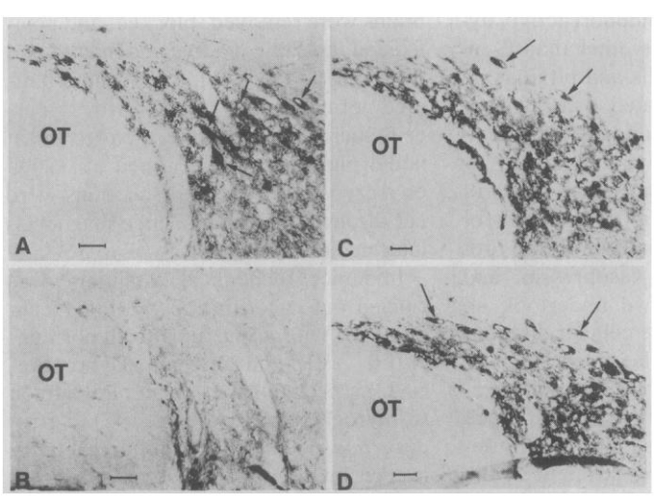
Two antiserums to AVP (1/1000 titer) and one antiserum to oxytocin (1/1000 titer) were also used in this study. By immunocytochemical standards all three magnocellular-directed antiserums were specific for their original peptide antigens. One set (prepared by G.N. and E.Z.) was produced by injecting rabbits with AVP or oxytocin coupled to bovine thyroglobulin with carbodiimide. These two antibodies stain different magnocellular neurons (*10*) and the staining can only be blocked by the homologous peptide; that is, AVP antiserum was blocked by 1 μ*M* AVP but not by oxytocin, β-endorphin, methionine-enkephalin, leucine-enkephalin, or dynorphin(1–13) up to concentrations of 50 μ*M*. Furthermore, the AVP antiserum did not stain the cells of homozygous Brattleboro rats.

The second AVP antiserum (1/1000 titer) (prepared by T.B. vW.G.) was produced by a similar conjugation strategy and resulted in a 0.6 percent cross-reactivity with oxytocin (at 50 percent displacement in a radioimmunoassay. In immunocytochemical tests 50 μ*M* oxytocin and the other peptides listed above could not block the cellular staining, whereas 1 μ*M* AVP completely blocked it (Fig. 1, C and D).

Two hundred serial sections (5 μm) were sequentially taken through the supraoptic and paraventricular nuclei of each of five rats. A series of 12 sections was stained as a unit with sections 1 and 12 being used for blockade of their respective antiserums with dynorphin(1–13) and AVP (or oxytocin). Sections 2, 4, 6, 8, and 10, for example, were stained for AVP and sections 3, 5, 7, 9, and 11 for dynorphin. Because of their size, the same magnocellular neurons were often found in three or more consecutive sections. For example, the cell might be stained in section 2 for AVP, section 3 for dynorphin, and section 4 for AVP again (Fig. 2A). A single cell was considered to contain both the dynorphin and vasopressin-like immunoreactivities if it stained for both compounds in at least three consecutive sections; and if the staining pattern was broadly observed across many cells, at several levels of the nucleus, and in tissue from several animals. We found that the two peptides generally, but perhaps not always, occur in the same cell (Fig. 2A).

By using the affinity-purified antibodies to dynorphin(1–13) we confirmed that the brain and pituitary regions contain dynorphin-like immunoreactivity (*4*). The posterior pituitary stained heavily whereas very little staining occurred in the intermediate lobe and only a few scattered cells stained in the anterior lobe. In the hypothalamus many cells in the supraoptic (Fig. 1A), paraventricular (Fig. 2B), and accessory nuclei showed positive staining. A few fibers stained in the median eminence. The staining of all of these cells was blocked by 1 μ*M* or 10 μ*M* dynorphin(1–13) (Fig. 1B) but not by the other peptides.

By using affinity-purified antiserum to leucine-enkephalin it is possible to show that many areas of rat brain contain enkephalin-positive cells (*11*). However, the magnocellular neurons in rat hypothalamus either do not stain or do so only very weakly (in contrast to other areas of the same section). Although the antiserum against leucine-enkephalin is blocked by 10 μ*M* dynorphin(1–13), it is possible, however, to visualize enkephalin-containing cells in the area of the paraventricular nucleus. Cells that are positive for dynorphin and AVP are not stained by antiserum to leucine-enkephalin under these conditions. Thus, in our experiments, leucine-enkephalin–positive cells and dynorphin-positive cells in the magnocellular nuclei are not the same.



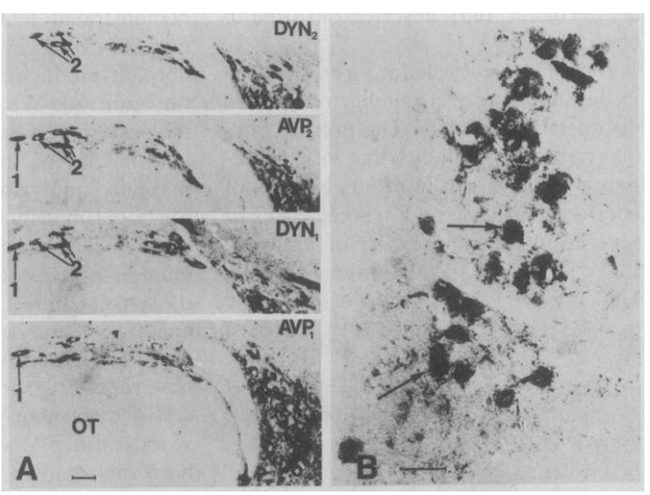


Fig. 1 (left). Adult male Sprague-Dawley rats received intracerebroventricular injections of colchicine (50 μg/50 μl) 48 hours before they were anesthetized and killed by perfusion. Serial sections through the supraoptic nucleus [next to the optic tract (*OT*)] were stained (A) with affinity-purified antibodies to dynorphin, (B) with the same antiserum plus 10 μ*M* dynorphin (control), (C) with antiserum to AVP, and (D) with the same antiserum to AVP plus 50 μ*M* oxytocin (arrows indicate darkly stained immunopositive cells). Fig. 2 (right). (A) Serial 5-μm sections through the supraoptic nucleus [near the optic tract (*OT*)] from a colchicine-treated rat (see legend to Fig. 1). Alternate sections were stained for AVP (AVP_1) then dynorphin (DYN_1) then AVP (AVP_2) and then again dynorphin (DYN_2). Several cells were stained in three consecutive sections (see sections 1 and 2). Section 1 shows a cell stained by antibodies to both AVP and dynorphin. Section 2 shows three such cells. Scale bar, 30 μm. (B) Dynorphin-positive cells (arrows indicate darkly stained immunopositive cells) in the paraventricular nucleus in homozygous Brattleboro rat. Scale bar, 30 μm.

After establishing that AVP and dynorphin occur in the same cells we repeated the study with oxytocin and dynorphin. In an equal number of parallel experiments with the same animals as studied for AVP and dynorphin we have detected no oxytocin in cells that contain dynorphin.

The homozygous Brattleboro rat produces no detectable quantities of AVP or its associated neurophysin (*6*). Therefore, it was of interest to determine whether dynorphin was present in the magnocellular cells of these animals and, if so, where. Using the same set of antiserums that was described above in five normal and five colchicine-treated homozygous Brattleboro rats, we were able to visualize cells containing oxytocin but no cells containing AVP. The dynorphin antiserums clearly showed positive cells in the supraoptic, circularis, and paraventricular nuclei (Fig. 2 B). The cells so visualized did not contain oxytocin. These data are in agreement with biochemical studies of the whole hypothalamus of Brattleboro rats (*7*). Our biochemical studies and those of other laboratories indicate that the amount of dynorphin in the brain of Brattleboro rats is the same as or slightly less than that in normal Sprague-Dawley rats.

Thus, the peptides dynorphin and vasopressin occur in the hypothalamus and pituitary in the same neurons. Furthermore, dynorphin occurs in non-oxytocin–containing neurons of the supraoptic nucleus of the homozygous Brattleboro rat. These data, taken with the approximate 300-fold molar excess of AVP over dynorphin (*2*, *7*), suggest that AVP and dynorphin are produced by genetically separate precursor systems.

Martin and Voight (*12*) reported enkephalin-like immunoreactivity in the posterior pituitary of the rat. They found immunoreactive methionine-enkephalin in oxytocin-containing fibers and immunoreactive leucine-enkephalin in vasopressin-containing fibers. However, they also reported that their leucine-enkephalin antiserum cross-reacted with dynorphin(1–13). Using affinity-purified antibodies to dynorphin(1–13) that do not cross-react with leucine-enkephalin, we have demonstrated dynorphin in vasopressin-positive cells and fibers. We suggest that dynorphin is at least one component of the "leucine-enkephalin–like immunoreactivity" found in vasopressin-producing cells and fibers. The present work does not address the issue of the relation of dynorphin to the putative enkephalin precursor (*13*). It does, however, suggest that dynorphin occurs in cells where leucine-enkephalin is not seen under the same conditions. This does not preclude a common biosynthetic origin, since peptides derived from a common precursor can be distributed or processed differently, as is the case for α-melanotropin in the intermediate lobe and adrenocorticotropin in the anterior lobe of pituitary (*14*). It is therefore possible that dynorphin and leucine-enkephalin either have separate precursors or have the same precursor but different post-translational events leading to their selective production in neurons of the central nervous system. However, biochemical evidence favors the possibility of separate precursors (*15*).

Glucagon-, cholecystokinin-, and angiotensin-II–like immunoreactivities have also been identified in magnocellular neurosecretory systems (*16*). The exact form of these peptides, their releasability, and their biological activity are open to question. Further, the biosynthetic relation of these substances to AVP, oxytocin, the neurophysins, and dynorphin is unknown. The possibility that one neuronal group synthesizes and releases several active neuropeptides from different biosynthetic pathways raises a variety of questions on the potential regulation of and interactions among these substances.

S. J. WATSON*
H. AKIL
Mental Health Research Institute, University of Michigan, Ann Arbor 48109

W. FISCHLI
A. GOLDSTEIN
Addiction Research Foundation, Department of Pharmacology, Stanford University, Palo Alto, California 94305

E. ZIMMERMAN
G. NILAVER
Department of Neurology, Columbia University, New York 10032

T. B. VAN WIMERSMA GREIDANUS
Rudolf Magnus Institute for Pharmacology, University of Utrecht, Utrecht, Netherlands

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* Send reprint requests to S.J.W.

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