regulation of the fluid and electrolyte balance.

We previously traced fibers from the area surrounding the anteroventral third ventricle to the portion of the parabrachial nucleus involved in cardiovascular regulation (*7*). We therefore prepared two additional rats by injecting fast blue dye, a retrograde fluorescent tracer, into the parabrachial nucleus 1 to 2 weeks before colchicine treatment. Sections through the preoptic area were stained by indirect immunofluorescence for APIr neurons. In each animal retrogradely labeled neurons were identified in the medial and periventricular preoptic nuclei in the area containing APIr neurons. When viewed with fluorescence illumination to demonstrate immunohistochemical staining, most of these retrogradely labeled neurons were also positive for APIr material (Fig. 1, H and I).

The presence of APIr neurons in the brain suggests that atriopeptins, like many other peptides, serve as central neurotransmitters or neuromodulators as well as hormones (*8*). The presence of APIr neurons in the anteroventral third ventricular region further suggests that atriopeptins modulate the same physiological responses systemically and in the central nervous system. Such a dual action has recently been proposed for corticotropin-releasing factor in cardiovascular regulation (*9*) and for cholecystokinin in the modulation of feeding (*10*).

Perhaps more apposite is an analogy with angiotensin II, which acts systemically to regulate blood pressure and electrolyte composition (*11*). Blood-borne angiotensin II is thought to enter the central nervous system through the subfornical organ, a circumventricular organ that lacks a blood-brain barrier (*12*). Subfornical organ neurons send projections into the hypothalamus that are believed to be involved in regulating blood volume and composition (*13*). Angiotensin II–like immunoreactivity was recently described in subfornical organ neurons, in which it may serve as a neurotransmitter (*14*). By comparison, APIr neurons in the periventricular preoptic area are adjacent to the organum vasulosum of the lamina terminalis, another circumventricular organ (*12*). It is possible that, in this location, they might be influenced by blood-borne factors, perhaps even systemic atriopeptins. Our finding that APIr neurons in the anteroventral periventricular preoptic nucleus innervate the part of the parabrachial nucleus involved in cardiovascular control supports the hypothesis that atriopeptin in the central nervous system plays a role in the regulation of blood volume and composition.

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4. Rats were perfused through the heart with 0.9 percent saline, followed by 200 ml of 4 percent paraformaldehyde in 0.1*M* phosphate buffer at *p*H 6.5, followed by an additional 200 ml of 4 percent paraformaldehyde in buffer at *p*H 8.5. The hearts and brains were removed and sectioned (50 μm) on a freezing microtome. Sections were incubated successively in phosphate-buffered saline (PBS) (0.1*M* phosphate buffer and 0.9 percent NaCl at *p*H 7.4) containing 0.25 percent Triton X-100 for 1 hour at 25°C; PBS containing 3 percent normal goat serum (PBS-G) for 1 hour at 25°C; PBS-G containing a 1:500 dilution of ATRP1 or ATRP7 or a 1:200 dilution of ATRP11 overnight at 4°C; and fluorescein isothiocyanate (FITC)– or horseradish peroxidase (HRP)–conjugated goat antiserum to rabbit immunoglobulin G diluted 1:50 in PBS-G for 1 hour at 25°C. Sections incubated in the FITC-labeled second antiserum were then mounted on gelatin-coated glass slides and cover slips were emplaced with glycerin. The slides were viewed with Leitz fluorescence illumination filter I2. Sections incubated with the HRP-labeled second antiserum were stained with 0.05 percent diaminobenzidine and 0.01 percent H_2O_2 in 0.1*M* phosphate buffer (*p*H 7.4) for 10 minutes, mounted, and dehydrated, and cover slips were emplaced with Histoclad.
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5 November 1984; accepted 17 December 1984

Distribution of Enkephalin Immunoreactivity in Germinative Cells of Developing Rat Cerebellum

Abstract. *The cellular distribution of enkephalin, an endogenous opioid, in the developing rat cerebellum was determined by immunocytochemistry. Methionine and leucine enkephalin were concentrated in the external germinal layer, a matrix of proliferative cells; staining was confined to the cortical cytoplasm. Enkephalin was not detected by immunocytochemistry in differentiated neural cells. These results indicate that endogenous opioids are involved specifically in early phases of nervous system development, particularly cell proliferation and differentiation.*

Endogenous opioid systems have been implicated in a wide variety of functions (*1*), including the regulation of nervous system development (*2, 3*). Endorphins are present in the plasma and brain tissue of developing organisms (*4–6*), and opiate receptors have been identified in brain (and body) tissues during ontogeny (*5, 7, 8*). The highest (and sometimes only) levels of opiate receptor binding and of tissue endorphins often occur during development (*5, 6, 8*). When infant rats receive the potent opioid antagonist naltrexone in a dosage regimen that

produces continuous or intermittent blockade of the interaction of endorphins and opiate receptors, somatic and neurobiological development is markedly altered (*2, 3, 9*). The effects on growth depend on the duration of opiate receptor blockade (*3*). For example, continuous blockade during early postnatal life dramatically increases body and brain size of young rats, increases the number of brain cells, and accelerates the appearance of physical characteristics and spontaneous motor and sensorimotor behaviors (*2, 3, 9*).

Enkephalin-like substances are detectable in fetal and neonatal cells by both immunocytochemistry and autoradiography (*10*). However, whether this enkephalin activity is unique to neuroblasts destined to be peptidergic neurons or is common to all proliferating neural cells has not been determined. If endogenous opioids alter neural cell proliferation or differentiation, endorphins should be associated with germinative brain cells but not necessarily with adult neural cells. To address this issue, we examined the distribution of enkephalin by immunocytochemistry in the cerebellum of postnatal rats. The cerebellum was selected because it has measurable endorphin levels and opiate binding activity only during its development (*5*). We identified enkephalin analogs in the external germinal layer, a transient cell matrix from which more than 90 percent of the cells of the cerebellar cortex arise during the first 3 weeks of postnatal life (*11*). Differentiated cerebellar neurons were not stained in the immunocytochemical reaction. These results provide evidence that endogenous opioids are involved in certain aspects of nervous system development.

Sprague-Dawley rats 10 to 35 days of age were anesthetized with ether and perfused intracardially with 4 percent paraformaldehyde in 0.07*M* Sorenson's phosphate buffer. Brains were removed immediately, postfixed for 90 minutes, and placed in 5 percent sucrose in 0.07*M* Sorenson's phosphate buffer at 4°C for 2 days or more. After immersing the tissue in Freon 12 we cut sections (20 μm) in a Slee cryostat and collected them on subbed cover glasses. Before immunocytochemical processing all sections were washed for 10 seconds in phosphate-buffered saline (PBS) at room temperature. Immediately before being stained with antibodies, some sections were incubated (60 minutes at 22°C) with $10^{-3}M$ Met-enkephalin or $10^{-3}M$ Leu-enkephalin (Sigma) in PBS containing 0.3 percent Triton X-100 (*12*). A wash procedure (*13*) was used to eliminate nonspecific binding. All sections were stained by indirect immunofluorescence with antiserum to Met-enkephalin or Leu-enkephalin (Immuno Nuclear). Primary antisera diluted 1:50 were utilized for sections that were not incubated in enkephalin solutions, whereas dilutions of 1:150 were used for incubated tissues. Sections were reacted with rhodamine-conjugated goat antibody to rabbit immunoglobulin G (1:50 dilution; Cappel Laboratories), mounted in glycerol, and viewed with Olympus optics. Control serum was prepared by absorbing antiserum to Met- or Leu-enkephalin with an excess of the respective antigen.

When we stained the cerebellums of 10-day-old rats with antiserum to Met-enkephalin and examined them at low magnification, we found that the intensity of fluorescence diminished progressively from the pial surface inward. The external germinal layer was brightly fluorescent (Fig. 1A), the molecular layer somewhat fluorescent, and the internal granular and medullary layers only slightly fluorescent. Higher magnification showed the perinuclear cytoplasm of the external germinal cells to be stained intensely but the nucleus to be only faintly fluorescent (Fig. 1B).

In the external germinal layer there was a gradient of fluorescence as well. Cells near the pial surface were stained more intensely than those adjacent to the molecular layer. The somata of Purkinje neurons and of glial cells in the medullary layer often displayed some immunofluorescence. The endothelial cells of blood vessels, particularly those in the molecular layer and pia, were usually stained intensely. Similar profiles of staining were observed when antiserum for Leu-enkephalin was used. Moreover, Met- and Leu-enkephalin immunoreactivity in sections incubated with enkephalins was identical to that in sections not subjected to such treatment. However, higher concentrations of antisera were needed to elicit staining of comparable intensity for nonincubated sections relative to incubated sections. The specificity of Met- and Leu-enkephalin immunoreactivity in the cerebellums of these rats was verified by inspection of control specimens (Fig. 1, C and D). The cerebellar cortex of 35-day-old animals showed little staining with antiserum to Met- and Leu-enkephalin (Fig. 1E), although some glial cell somata and blood vessels did fluoresce weakly.

Our results show that enkephalin-like substances are present in the developing

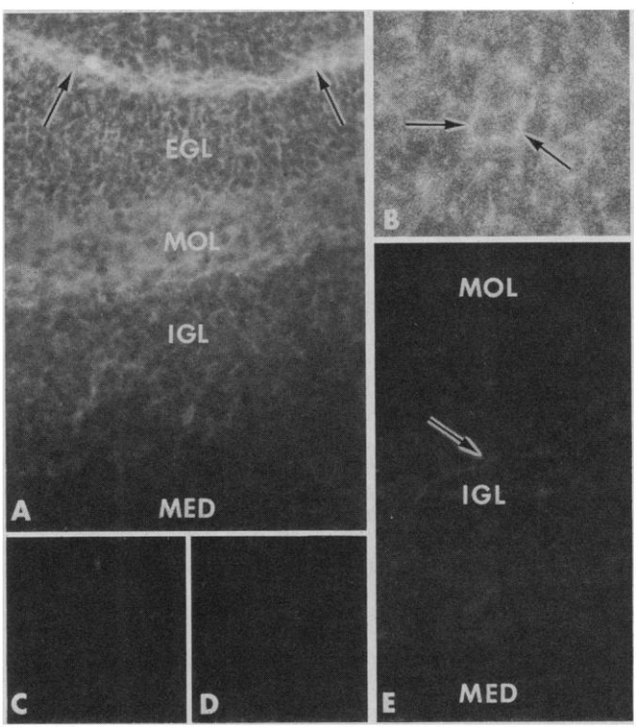

Fig. 1. (A to E) Photomicrographs showing the location of Met-enkephalin immunoreactivity in the cerebellum of 10- and 35-day-old rats. (A) Cerebellum of a 10-day-old rat (×215). Note the bright staining of the external germinal layer (EGL) and the low to moderate staining of the molecular layer (MOL), internal granule layer (IGL), and medullary layer (MED). Blood vessels in the pia (arrows) and molecular layer are often extremely fluorescent. (B) External germinal cells of the external germinal layer (×815). Note the intensely stained cortical cytoplasm (arrows) encircling a nonfluorescent nucleus. (C and D) Control sections stained with Met-enkephalin antiserum absorbed with Met-enkephalin (C) or stained with serum from unimmunized animals (D). Both photomicrographs (×215) are of the external germinal layer (identified by phase optics) and times of exposure and printing correspond to those in (A). (E) Cerebellum of a 35-day-old rat (×215). Except for lightly fluorescent endothelial cells of blood vessels (arrow), little staining was observed. The times of exposure and printing are similar to those in (A).

rat cerebellum, particularly the external germinal layer. Enkephalin immunoreactivity is especially intense in germinative neural cells but can also be detected in other neuronal and glial cell types at early postnatal ages. Furthermore, the enkephalins have a specific subcellular location and are associated with the cortical cytoplasm but not the cell nucleus. Finally, the presence of enkephalin-like material in the cerebellum is age-dependent; the highest activity is detected during the periods of most rapid cerebellar development.

Our finding that enkephalin-like substances are present in the rat cerebellum only during development is consistent with earlier reports in which the greatest concentrations of β-endorphin and enkephalin were found during the first two postnatal weeks (*5, 6*). The endorphins are known to interact with specific brain receptors (*1*). In the cerebellum the highest levels of tritiated naloxone and tritiated Met-enkephalin binding also occur during the first 2 weeks (*5*). We have found that the major location of enkephalin-like activity in the cerebellum of postnatal rats is the external germinal layer. This proliferative cell matrix generates microneurons over the first 3 postnatal weeks, with a crescendo of activity occurring at 6 to 10 days. On the basis of previous in vivo and in vitro studies on the action of endogenous and exogenous opioids and growing cells and organisms (*2, 3, 9, 14, 15*), it appears that the endorphins interact with opiate receptors on developing cells in such a way as to inhibit cell proliferation; their effect on cell migration and differentiation is unclear (*2, 3, 15*). The presence of enkephalin immunoreactivity in developing but not adult nervous tissues indicates that endogenous opioids are involved in specific aspects of nervous system development.

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29 October 1984; accepted 5 December 1984

Electrical Sources in Human Somatosensory Cortex: Identification by Combined Magnetic and Potential Recordings

Abstract. *Magnetic fields and electrical potentials produced by neuronal activity have different properties that can be used for the identification of electrical sources in the human brain. Fields and potentials occurring 20 to 30 milliseconds after median nerve stimulation in human subjects were compared in order to investigate the sources of evoked potential components that have been attributed by different investigators to the thalamus or thalamocortical afferents, to separate radial sources in somatosensory cortex and motor cortex, or to a tangential source in somatosensory cortex. The magnetic and potential wave forms were highly similar in morphology, and their spatial distributions were centered over sensorimotor cortex, were dipolar in shape, and differed in orientation by approximately 90 degrees; distances between the minimum and maximum of the magnetic distributions were about 60 percent of those of the potential distributions. These results cannot be accounted for by thalamic sources or radial cortical sources alone, but are consistent with a tangential source in somatosensory cortex, with an additional smaller contribution from radial sources.*

Stimulation of the human median nerve at the wrist elicits a series of evoked potentials (EP's) that can be recorded noninvasively from the head and neck and are widely used to assess neurological abnormalities in the somatosensory pathway. The anatomical sources for some of these potentials are established but there is disagreement about others (*1*). We have used the different properties of magnetic and potential recordings to test alternative hypotheses for the sources of EP's at 20 and 30 msec, the origins of which are controversial.

The 20- and 30-msec EP's are seen at scalp locations contralateral to the nerve stimulated (*2, 3*). Evoked potentials at parietal locations are negative to positive (N20-P30), whereas those at frontal locations have peaks at similar latencies but opposite polarities (P20-N30). Evoked potentials with similar wave forms and larger amplitudes are seen in cortical surface recordings, with N20-P30 maximal in the hand representation area of somatosensory cortex and P20-N30 maximal in the hand area of motor cortex (*4, 5*). These distributions and recordings in patients with thalamic or cortical damage have led to three hypotheses concerning sources for N20 and P20 (*6*): (i) thalamus or thalamocortical afferents (*7, 8*); (ii) a pair of radially oriented sources in somatosensory cortex and the motor cortex (*3, 9*); and (iii) a single tangentially oriented source in area 3b of somatosensory cortex (*4, 5, 10*).

Magnetic recordings of brain activity can be made with devices capable of detecting the extremely small magnetic fields produced by neuronal currents (*11*). Theory indicates that surface magnetic and potential distributions have different properties that can be used for source identification (*11–13*): (i) Whereas potential recordings are sensitive to both tangential and radial current sources, magnetic recordings are sensitive only to that portion of the source having a tangential orientation to the scalp. (ii) For tangential dipoles, the surface magnetic distribution is oriented at right angles to the potential distribution. (iii) Unlike potential recordings, magnetic recordings are uninfluenced by the skull and scalp, and this results in magnetic distributions with a smaller spatial extent. Because of these different properties, the three source hypotheses lead to different predictions about the magnetic and potential distributions for the 20- to 30-msec potentials (*6*). We report direct comparisons of magnetic and potential record-