by chromosomal translocation, either of which results in the juxtaposition of the *tcl*-1 gene and the α-chain locus (Fig. 4).

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References and Notes

1. J. P. Allison *et al.*, *J. Immunol.* **129**, 2293 (1982); K. Haskins *et al.*, *J. Exp. Med.* **157**, 1149 (1983); S. C. Meuer *et al.*, *ibid.*, p. 705; L. E. Samelson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6972 (1983); J. Kaye *et al.*, *J. Exp. Med.* **158**, 836 (1983).
2. S. M. Hedrick *et al.*, *Nature (London)* **308**, 149 (1984); S. M. Hedrick *et al.*, *ibid.*, p. 153.
3. Y. Yanagi *et al.*, *ibid.*, p. 145.
4. Y. Chien *et al.*, *ibid.* **312**, 31 (1984).
5. H. Saito *et al.*, *ibid.*, p. 36.
6. Y. Chien *et al.*, *ibid.* **309**, 322 (1984); G. Siu *et al.*, *ibid.* **311**, 344 (1984); J. Kavaler *et al.*, *ibid.* **310**, 421 (1984).
7. N. Caccia *et al.*, *Cell* **37**, 1091 (1984).
8. C. M. Croce *et al.*, *Lab. Invest.* **51**, 258 (1984).
9. D. Morgan *et al.*, *Science* **193**, 1007 (1976).
10. J. Erikson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 820 (1983).
11. J. Erikson and C. M. Croce, unpublished results.
12. C. M. Croce *et al.*, *Nature (London)* **249**, 839 (1974); C. M. Croce *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3416 (1979).
13. B. S. Emanuel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2444 (1984).
14. D. L. Williams *et al.*, *Cell* **36**, 101 (1984).
15. Y. Ueshima *et al.*, *Blood* **63**, 1028 (1984).
16. L. Zech *et al.*, *Int. J. Cancer* **32**, 431 (1981); L. Zech *et al.*, *Nature (London)* **308**, 858 (1984); F. Hecht *et al.*, *Science* **226**, 1445 (1984).
17. Y. Tsujimoto *et al.*, *Science* **224**, 1403 (1984); *ibid.* **226**, 1097 (1984).
18. B. K. McCaw *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 207 (1975).
19. R. Dalla-Favera *et al.*, *ibid.* **79**, 7824 (1982); C. M. Croce *et al.*, *ibid.* **80**, 6922 (1983); J. Erikson *et al.*, *ibid.*, p. 7581.
20. V. Gubler and B. J. Hoffman, *Gene* **25**, 263 (1983).
21. J. Vieira and J. Messing, *ibid.* **19**, 259 (1982); D. M. Helfman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 31 (1983).
22. D. Hanahan, *J. Mol. Biol.* **166**, 557 (1983).
23. M. Grunstein and D. S. Hofner, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3961 (1975).
24. F. Sanger *et al.*, *ibid.* **74**, 5463 (1977); J. Messing, *Methods Enzymol.* **101**, 20 (1983).
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Atriopeptin-Immunoreactive Neurons in the Brain: Presence in Cardiovascular Regulatory Areas

Abstract. *Antisera to atriopeptin III and to a cyanogen bromide fragment of the precursor molecule atriopeptigen were prepared and used to examine the distribution of atriopeptin-like immunoreactive material in the heart and brain of the rat. Granules of this material were seen in myocytes throughout the right and left atria and were densest in the perinuclear region. The distribution of atriopeptin-like immunoreactive material in the heart is consistent with previous reports of atrial secretory granules. In the brain neurons containing the material were observed in the hypothalamus and the pontine tegmentum. Atriopeptin in the brain may serve as a neurotransmitter in neural systems controlling blood volume and composition, the same physiological functions regulated by blood-borne atriopeptin.*

It has been known for over 20 years that atrial myocytes contain secretory granules, but the function of these granules has remained obscure. Recently, peptides isolated from atria have been shown to have natriuretic and smooth muscle relaxant effects (*1*). Several of these atrial peptides (atriopeptins) have been purified and their amino acid sequences determined (*2*). The availability of synthetic atriopeptins and an isolated purified fragment of the high molecular weight precursor has allowed preparation of antisera for use in radioimmunoassays and immunohistochemistry. Using the latter method, we found that atriopeptin-like immunoreactivity is present in the atria in a pattern that implies its presence in atrial secretory granules. We also identified atriopeptin-like immunoreactive (APIr) neurons in the brain.

Two different antisera to synthetic atriopeptin III (AP III) and one antiserum to a high molecular weight cyanogen bromide fragment of the atriopeptigen precursor (HMW AP) were prepared (*3*). The specificity of these antisera was examined by measuring the displacement of [^{125}I]AP III by unlabeled AP III. Both antisera to AP III showed 100 percent cross-reactivity with biologically active atriopeptins I and II (*2*). However, neither showed significant cross-reactivity with two biologically inactive atriopeptin analogs: (i) the fragment of AP III consisting of amino acid residues 13 to 24 and (ii) AP III containing an inversion of the arginine-isoleucine sequence at positions 7 and 8. The antiserum to HMW AP was 100 percent cross-reactive with atriopeptins I and II. Ten percent of the binding of [^{125}I]AP III was displaced by equimolar concentrations of HMW AP, but the biologically inactive atriopeptin analogs were without effect.

The two antisera to AP III (ATRP1 and ATRP7) and one antiserum to HMW AP (ATRP11) were used for immunohistochemical staining of the heart (*4*). Although the staining with ATRP11 was heaviest, the pattern of APIr staining was confirmed with the two AP III antisera. There was staining of granular material in myocytes throughout the right and left atria; the granular immunoreactive material was densest in the perinuclear region (Fig. 1A). No staining was seen in the ventricle (Fig. 1, C to D). Atrial staining by ATRP11 was abolished by preadsorbing 1 ml of the diluted antiserum with 50 μg of HMW AP (Fig. 1B) and was partially blocked by preadsorption with synthetic AP III, but was not blocked by AP III-(13–24) or by AP III containing the inversion. Staining with AP III antisera was completely blocked by preadsorption with synthetic AP III but was not affected by preadsorption with either of the biologically inactive atriopeptin analogs, and was only partially inhibited by keyhole limpet hemocyanin (KLH) (*3*).

The granular distribution of APIr staining in the heart was consistent with the localization of atriopeptins in secretory granules in atrial myocytes (*1*). In electron micrographs secretory granules tend to be localized most densely in the perinuclear region of these cells (*1*), and APIr granules in our material matched this distribution. We conclude that our antisera were probably staining immunoreactive atriopeptins in atrial secretory granules.

The brain was examined for APIr staining in a series of 11 rats. In one animal, which had not been treated with colchicine, two main groups of APIr fibers were visualized with the antiserum to HMW AP. In the hypothalamus labeled fibers extended from the periventricular nucleus into the medial preoptic area, the ventral and lateral parts of the bed nucleus of the stria terminalis, the lateral hypothalamic area, and the paraventricular and arcuate nuclei. A second heavily stained bundle of fibers was seen in the pons emerging from the periventricular gray matter of the fourth ventri-

cle. These fluorescent axons took a paramedian course rostrally and ventrally to innervate the dorsal and lateral divisions of the interpeduncular nucleus. No staining of neuronal perikarya was seen in this brain, and neither cell nor fiber staining was seen in similar material from another rat stained with the AP III antisera. The remaining rats received colchicine (150 to 300 μg, intraventricularly) 27 to 40 hours before being killed to block axoplasmic transport and increase peptide content in neuronal cell bodies (*5*). The heaviest staining was once again achieved with the HMW AP antiserum but was confirmed with the two AP III antisera. Control incubations in which the HMW AP antiserum had been preadsorbed with HMW AP, or in which either of the AP III antisera had been preabsorbed with synthetic AP III, showed no neuronal staining. Prior incubation with either of the biologically inactive AP III analogs did not affect the staining. Adsorption of HMW AP antiserum with AP III, or of AP III antisera with KLH (*3*), resulted in diminished intensity of staining.

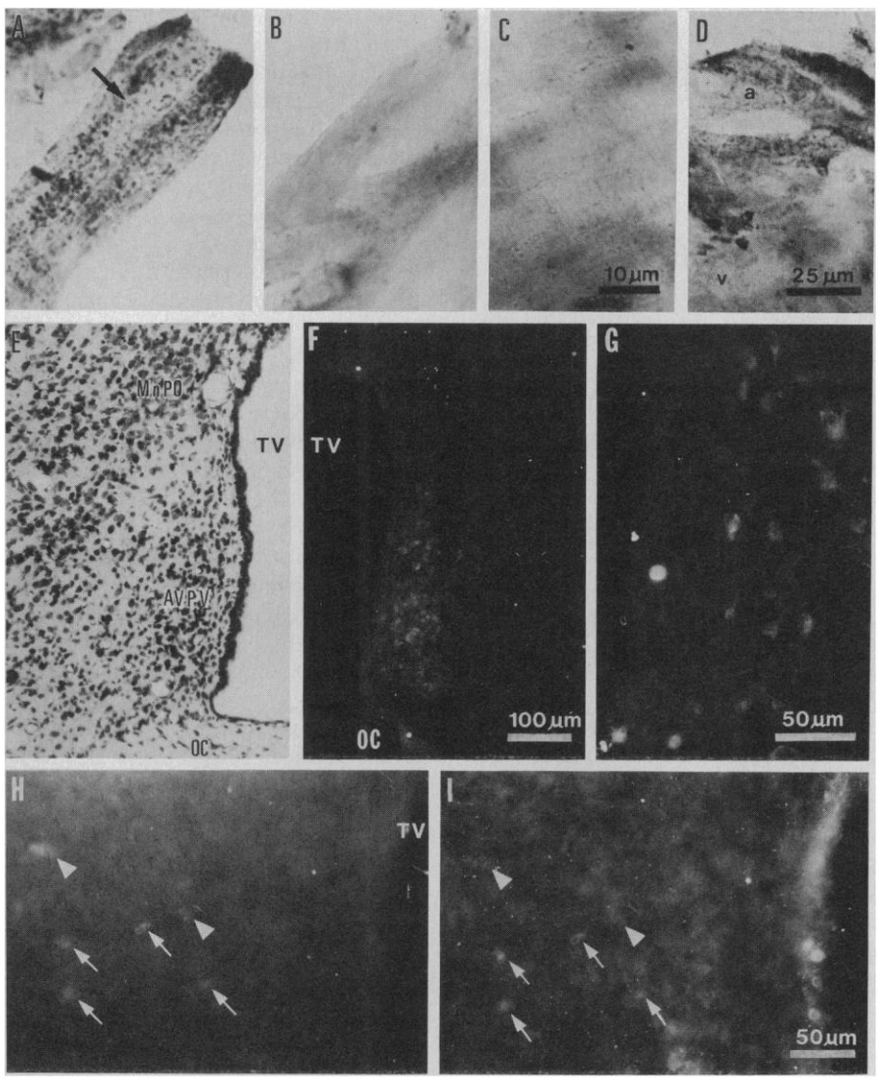

Fig. 1. Photomicrographs showing the appearance of immunohistochemical staining of the heart and the brain for AP III with antiserum ATRP1. (A) Granular appearance of APIr material in myocytes from the right atrium. The nucleus of one myocyte is seen as an unstained oval area (arrow) surrounded by a dense cluster of labeled granules. (B) Absence of granular label in a similar atrial preparation in which the ATRP1 antiserum was adsorbed with AP III. (C) Section through the left ventricle stained for atriopeptin; the muscle striations are faintly seen but no granular material is stained. The contrast between staining of atrium and ventricle is seen in (D), which shows the atrioventricular junction. Atrial myocytes (a) with granular staining are seen juxtaposed on the same section with ventricular tissue (v) that does not stain. (E) Bright-field appearance of a thionin-stained section through the preoptic area (MnPO, median preoptic area; OC, optic chaism; TV, third ventricle). The anteroventral periventricular preoptic nucleus (AVPV) is a distinct cell cluster that corresponds to the APIr cell group seen in a fluorescence photomicrograph of a similar section from the contralateral side of another brain (F). (G) Higher magnification view of the AVPV APIr neurons. (H and I) Fluorescence photomicrographs of a section through the AVPV, illustrating six neurons retrogradely labeled with fast blue after the injection into the parabrachial nucleus; four of these neurons are stained immunohistochemically for AP III (arrows) while two others are not (arrowheads). The photograph in (H) was taken with a Leitz A filter and the photograph in (I) with a Leitz I2 filter.

Immunohistochemically stained neuronal cell bodies were found in two regions. The largest collection of APIr neurons was found in the hypothalamus. A dense cluster of small, bipolar APIr neurons was seen in the anteroventral periventricular preoptic nucleus adjacent to the wall of the most anteroventral extreme of the third ventricle (Fig. 1, E to G). Immunoreactive perikarya were found extending laterally into the medial preoptic area, the preoptic part of the bed nucleus of the stria terminalis, and the ventral pallidum. More caudally, APIr neurons were seen along the wall of the third ventricle, in the medial parvocellular part of the paraventricular nucleus, and in the periventricular and arcuate nuclei. Other APIr neurons extended laterally, bordering the dorsal and ventral edges of the paraventricular nucleus, and invaded the perifornical region. A separate collection of larger, multipolar APIr cell bodies was seen in the lateral hypothalamic area as far caudally as the premammillary region.

The second region containing APIr neurons was the pons. Rostrally, these large multipolar neurons were scattered in the periaqueductal gray matter just lateral to the dorsal raphe nucleus. As the cerebral aqueduct opened into the fourth ventricle, APIr neurons became more numerous, forming a cluster in the laterodorsal tegmental nucleus at the lateral corner of the periventricular gray matter. Some APIr neurons spilled laterally into the pedunculopontine nucleus, surrounding the superior cerebral peduncle at this level. More caudally, as the locus ceruleus became more prominent, APIr neurons were found in the paramedian area of the floor of the fourth ventricle, surrounding the dorsal tegmental nucleus, and scattered in the remaining periventricular gray matter.

The distribution of APIr neurons in the anteroventral third ventricular region is of special interest, as lesions in this area have profound effects on fluid and electrolyte balance in rats (*6*). Animals with lesions do not spontaneously drink sufficient quantities of fluids and become hypovolemic and hypernatremic. The identification of the neural connections of the APIr neurons will be useful in determining whether they participate in

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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References and Notes

1. J. D. Jamieson and G. E. Palade, *J. Cell Biol.* **23**, 151 (1964); A. J. DeBold, *Proc. Soc. Exp. Biol. Med.* **161**, 508 (1979); M. G. Currie *et al.*, *Science* **221**, 71 (1983); M. Cantin *et al.*, *Histochemistry* **80**, 113 (1984).
2. M. G. Currie *et al.*, *Science* **223**, 67 (1984); D. M. Geller *et al.*, *Biochem. Biophys. Res. Commun.* **121**, 802 (1984); D. M. Geller *et al.*, *ibid.* **120**, 353 (1984); G. Thibault *et al.*, *FEBS Lett.* **167**, 352 (1984); K. Kangawa *et al.*, *Biochem. Biophys. Res. Commun.* **119**, 933 (1984); T. G. Flynn, M. L. deBold, A. J. deBold, *ibid.* **117**, 859 (1983).
3. J. L. Vaitukaitis, *Methods Enzymol.* **73**, 46 (1981). Twenty-five rabbits were injected intradermally with 500 μg of KLH conjugated to AP III (KLH–AP III) in complete Freund's adjuvant. Subsequent boosts of 100 μg of KLH–AP III were given monthly for 4 to 6 months. Sera with significant binding activity to [^{125}I]AP III were evident by the fourth month in 3 of 25 rabbits; the two with the highest titers (ATRP 1 and ATRP 7) were used for immunohistochemistry. Four rabbits were injected intradermally with 100 μg of HMW AP, a 9500-dalton cyanogen bromide fragment of the atriopeptigen precursor containing the 92 carboxyl terminal amino acids. Each rabbit received a single boost of 50 μg of HMW AP at 3 months. Only one rabbit (ATRP 11) showed significant binding titers for AP III.
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.
5. T. Hokfelt and A. Dahlstrom, *Z. Zellforsch. Mikrosk. Anat.* **114**, 460 (1971).
6. M. J. Brody and A. K. Johnson, *Disturbances in Neurogenic Control of the Circulation* (American Physiological Society, Rockville, Md., 1981), pp. 105–117.
7. C. B. Saper and D. Levisohn, *Brain Res.* **288**, 21 (1983); R. B. Hamilton *et al.*, *J. Auton. Nerv. Syst.* **4**, 261 (1981); J. Mraovich, M. Kumada, D. J. Reis, *Brain Res.* **232**, 57 (1982).
8. D. T. Krieger, *Science* **222**, 975 (1983).
9. L. W. Swanson, P. E. Sawchenko, J. Rivier, W. W. Vale, *Neuroendocrinology* **36**, 165 (1983); L. A. Fisher and M. R. Brown, *Brain Res.* **296**, 41 (1984).
10. M. L. McCaleb and R. D. Myers, *Peptides* **1**, 47 (1980); J. Maddison, *Physiol. Behav.* **19**, 819 (1977); C. Fulwiler and C. B. Saper, *Neurosci. Lett.*, in press.
11. W. C. Bowman and M. J. Rand, *Textbook of Pharmacology* (Blackwell, Oxford, 1980), pp. 12.28–12.31.
12. J. B. Simpson, *Neuroendocrinology* **32**, 248 (1981); M. L. Mangiapane and J. B. Simpson, *Am. J. Physiol.* **239**, R382 (1980); R. D. Broadwell and M. W. Brightman, *J. Comp. Neurol.* **166**, 257 (1976).
13. R. W. Lind, G. W. van Hoesen, A. K. Johnson, *J. Comp. Neurol.* **210**, 265 (1982); R. R. Miselis, R. E. Shapiro, P. J. Hand, *Science* **205**, 1022 (1979); R. R. Miselis, *Brain Res.* **230**, 1 (1981).
14. R. W. Lind, L. W. Swanson, D. Ganten, *Brain Res.* **321**, 209 (1984).
15. We thank K. Fok for preparing AP III–KLH, Quan Hue Ha for technical assistance, and P. Nacci for secretarial help. Supported by Monsanto Corp. and PHS grants NS18669 and GM07200 (Medical Scientist). C.B.S. is the recipient of National Institute of Neurological and Communicative Disorders and Stroke Teacher-Investigator Development Award NS00631 and a McKnight Scholar Award.

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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