4. ———, *Biochem. Pharmacol.* **24**, 495 (1975).
5. S. R. Butler and S. M. Schanberg, *Trans. Am. Soc. Neurochem.* **8**, 259 (1977); *Life Sci.* **21**, 877 (1977).
6. M. A. Hofer, *Science* **168**, 871 (1970).
7. ———, *ibid.* **172**, 1039 (1971).
8. Radioactive ornithine was obtained from New England Nuclear.
9. M. A. Hofer and H. Weiner, *Psychosom. Med.* **33**, 353 (1971).
10. J. Bartolomé, C. Lau, T. Slotkin, *J. Pharmacol. Exp. Ther.*, in press.
11. D. W. Lincoln and J. B. Wakerley, *J. Physiol. (London)* **242**, 533 (1974).
12. G. Sayers, *Physiol. Rev.* **30**, 241 (1950).
13. This work was supported by NIH grant MH-13688 and NIH research scientist award MH-06489 to S.M.S.

19 May 1977; revised 22 August 1977

A Nonpeptide Morphine-Like Compound: Immunocytochemical Localization in the Mouse Brain

Abstract. *A nonpeptide morphine-like compound (MLC) which cross reacts with morphine-specific antibodies has been localized with the use of immunocytochemistry. This morphine-like compound is found in neuronal perikarya or processes (or both) in nuclei related to vestibular, cerebellar, and raphe systems.*

The nervous system contains endogenous compounds which in their actions resemble opiates (*1*). The most investigated of these compounds are the peptides such as enkephalin or endorphins. However, there is another type of endogenous morphine-like compound (MLC) which is not a peptide and which has been called MLC (*2*). In addition to binding to opiate receptors (*3*), MLC also binds to antibodies generated against morphine haptene (*2*). Critical to developing an understanding of the function of MLC is the determination of the type and distribution of the cells that contain it. We now report the localization of MLC in the perikarya and processes of neurons in nuclei related to vestibular, cerebellar, and raphe systems.

Rabbit antiserum containing antibodies directed specifically against morphine, first described by Spector (*4*), was used in these studies. Mice were killed by cervical dislocation, and the brains were removed and fixed for 4 hours in Bouin's reagent. The tissue was then dehydrated through a graded series of ethanol solutions, embedded in paraffin, and cut at 6 μm. Sections of fixed brain were then deparafinized, taken to water, and incubated with rabbit antiserum to morphine haptene for 72 hours in a humid atmosphere at 4°C. The antibody was localized by the unlabeled PAP (peroxidase-antibody to peroxidase) technique (*5*). For this technique, the sections were first incubated with the antiserum to morphine (diluted 1 : 500 in 0.1*M* phosphate buffered saline at *p*H 7.4), and rinsed twice in buffer for 5 minutes with constant shaking. The sections were then incubated (i) with sheep antiserum (diluted 1 : 100) to rabbit globulin for 30 minutes, (ii) treated by the PAP technique (antibody to peroxidase dilution, 1 : 200) for 1 hour, (iii) and then with 3,3′-diaminobenzidine (50 mg/100 ml) with 0.003 percent hydrogen peroxide for 6 minutes with agitation. The sections were rinsed in distilled water, dehydrated through a graded series of ethanol solutions and mounted in Permount. All solutions containing serums were passed through a Millipore (0.2 mm) filter just before use.

Controls consisted of serial sections to the ones exposed to specific antibody, but incubated instead with nonimmune serum, or morphine antiserum from which specific antibody had been removed by passage over a column containing morphine immobilized on Sepharose. No reactive neurons or processes were seen in these sections.

Several regions of the brain stem contained neurons reactive to MLC. One area showing intense immunoreactivity for MLC surrounded (within 0.2 mm) the floor of the caudal half of the fourth ventricle. There was much reaction product in neuronal perikarya and neurites in the medial vestibular nucleus and the praepositus hypoglossi (Fig. 1). Ependymal cells of the choroid plexus are not illustrated, but they also showed intense staining. Figure 2 shows staining of neuronal perikarya in the median raphe in the vicinity of the nucleus raphe magnus. Neurons and neuropil in the nucleus gigantocellularis also contained considerable reaction product (Fig. 3).

Other areas (not illustrated) in which reactive cells were seen included the vermis of the cerebellum, the deep cerebellar nuclei (for example, the dentate, fastigial, and interpositus, the nucleus of Roller, nucleus lateralis, medulla oblongata (*6*), and area postrema of the medulla.

Using techniques of light microscopic immunocytochemistry we have succeeded in localizing in both neuronal perikarya and processes a compound that cross reacts with specific antibody to morphine. The presence of this material in these structures suggests that it might play an important role in neuronal function. The presence of MLC in discrete nuclei further suggests that it might be associated with a restricted number of neuronal groups.

The localization of MLC in various brain stem nuclei could imply its involvement with certain neuronal pathways associated with stimulus produced analgesia (SPA). It has been well established that stimulation of medial brain stem structures such as periventricular structures and caudal raphe nuclei pro-

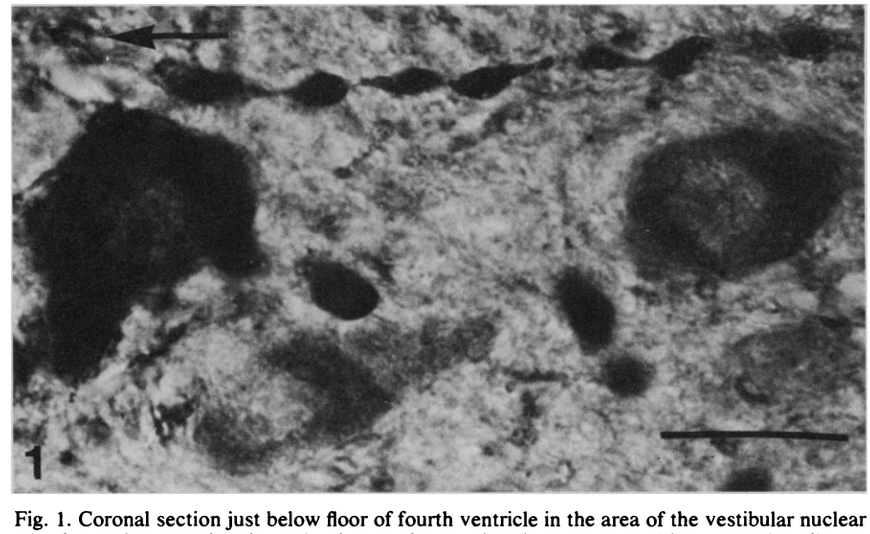
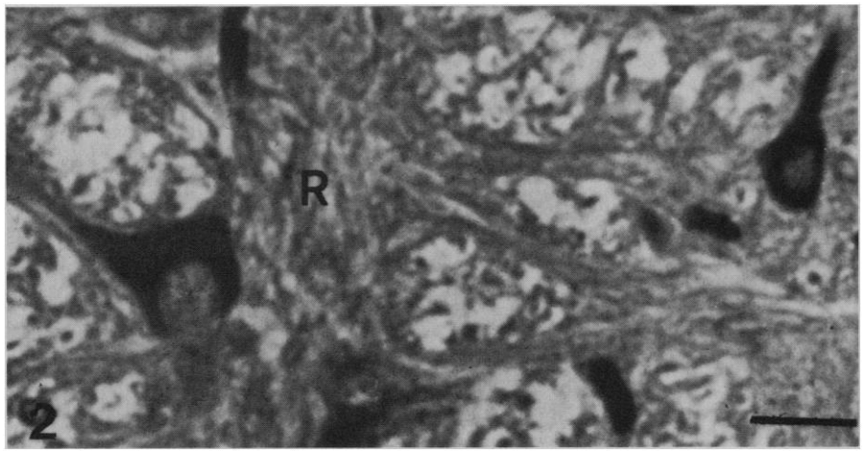


Fig. 1. Coronal section just below floor of fourth ventricle in the area of the vestibular nuclear complex and praepositus hypoglossi. Reaction product is concentrated in neuronal perikarya and in a varicose axon running across the upper portion of the field. Although reaction product is most intense in the varicosities, staining of thin intervaricose segments can also be seen. Arrow points in the direction of the fourth ventricle (scale bar, 20 μm).

0036-8075/78/0127-0447$00.50/0

duces analgesic responses (*7*). In this study, MLC has been visualized immunohistochemically in both these regions. In addition, stimulation of the periaqueductal gray in the rat has been shown to suppress the nociceptive responses of neurons in nucleus gigantocellularis of the medullary reticular formation (*8*). This area also is distinguished by neurons that contain MLC. Reactive cells were also observed in cells of the vermis and the fastigial and interpositus nucleus of the cerebellum. This fact alone is interesting in view of the noticeable absence of enkephalin in the cerebellum (*9*) and since stimulation of cerebellar structures in monkey can alter nociceptive responses (*10*). Moreover, it has been shown that there are projections from the vermis to neurons in the fastigial nucleus, which in turn project to gigantocellularis (*11*). In addition, the raphe has projections to cells in the vermis (*12*). There is also a reciprocal projection from the vestibular complex and the perihypoglossal nuclei to the vermis and the fastigial nucleus (*13*). Since MLC has been found in all of these regions, it could have a role in the regulation of these pathways which, because of their interconnections, could be called a vestibular-raphe-cerebellar system.



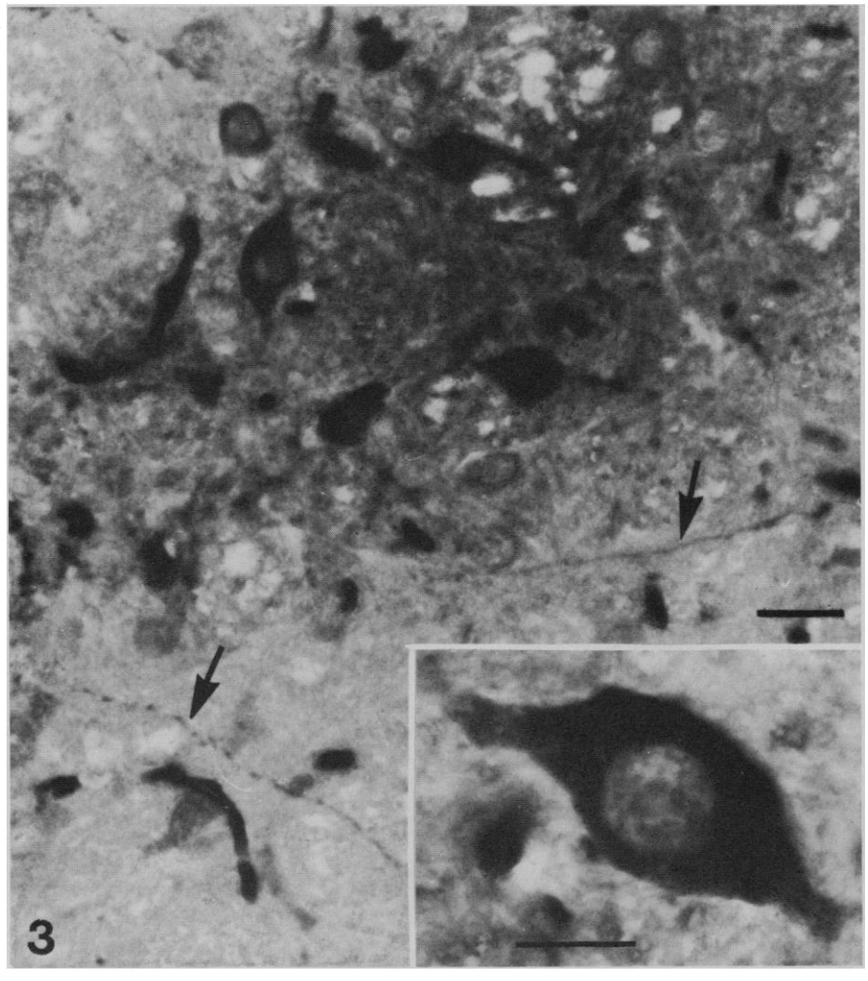


Fig. 2. Coronal section near the nucleus raphe magnus. Note reaction product in neuronal perikarya near the midline and paramedian raphe (*R*, raphe; scale bar, 20 μm). Fig. 3. Coronal section from the region of the nucleus gigantocellularis. The reaction product is in clusters of neuronal perikarya and the surrounding neuropil. The arrows point to positive neurites (scale bar, 20 μm, inset scale bar, 10 μm).

ALAN R. GINTZLER
MICHAEL D. GERSHON
Department of Anatomy, Columbia University, College of Physicians and Surgeons, New York 10032
SYDNEY SPECTOR
Roche Institute of Molecular Biology, Nutley, New Jersey 07110

References and Notes

1. J. Hughes, *Brain Res.* **88**, 295 (1975); G. W. Pasternak, R. Goodman, S. Snyder, *Life Sci.* **16**, 1765 (1975); J. Hughes, T. W. Smith, H. W. Kosterlitz, L. Fothergill, B. A. Morgan, H. R. Morris, *Nature (London)* **258**, 578 (1975); T. Terenius and A. Wahlstrom, *Life Sci.* **16**, 1759 (1975); H. Teschemacher, K. E. Opheim, B. M. Cox, A. Goldstein, *ibid.*, p. 1771; R. Simantov and S. Snyder, *ibid.* **18**, 781 (1976); B. M. Cox, A. Goldstein, C. H. Li, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 182 (1976); N. Ling, R. Burgus, R. Guillemin, *ibid.*, p. 3942.
2. A. Gintzler, A. Levy, S. Spector, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2132 (1976).
3. A. Blume, J. Shorr, J. Finberg, S. Spector, *ibid.* **74**, 4927 (1977).
4. S. Spector and C. W. Parker, *Science* **168**, 1347 (1970); S. Spector, *J. Pharmacol. Exp. Ther.* **178**, 253 (1971).
5. L. A. Sternberger, *Electron Microscopy of Enzymes, Principles and Methods* (Van Nostrand, New York, 1973), pp. 150–181.
6. R. L. Sidman, J. B. Angevine, Jr., E. T. Pierce, *Atlas of the Mouse Brain and Spinal Cord* (Harvard Univ. Press, Cambridge, Mass., 1971), pp. 88–96.
7. D. Richardson and H. Akil, *J. Neurosurg.*, in press; T. D. Oleson and J. C. Liebeskind, *Physiologist* **18**, 338 (1975); H. K. Proudfit and E. G. Anderson, *Brain Res.* **98**, 612 (1975); J. L. Oliveras, F. Redjemi, G. Guilbaud, J. M. Besson, *Pain* **1**, 139 (1975); D. J. Mayer and D. D. Price, *ibid.* **2**, 379 (1976).
8. T. J. Morrow and K. L. Casey, *Proceedings of the First World Congress on Pain* (Raven, New York, in press).
9. J. Hughes, *Brain Res.* **88**, 295 (1975); G. W. Pasternak, R. Simantov, S. Snyder, *Mol. Pharmacol.* **12**, 504 (1975).
10. P. Siegel and J. G. Nepsic, *Physiol. Behav.* **13**, 189 (1974).
11. D. R. P. Eager, *J. Comp. Neurol.* **120**, 81 (1963); M. B. Carpenter, D. S. Bard, F. A. Alling, *ibid.* **111**, 1 (1959); M. B. Carpenter, *Am. J. Anat.* **104**, 1 (1959); F. Walberg, O. Pompeiano, A. Brodal, J. Jansen, *J. Comp. Neurol.* **118**, 49 (1962).
12. R. L. M. Faull, *Anat. Rec.* **187**, 577 (1977).
13. A. Torvik and A. Brodal, *J. Neurophysiol. Exp. Neurol.* **13**, 515 (1954); F. Walberg, *Exp. Neurol.* **3**, 525 (1961); P. Angaut and A. Brodal, *Arch. Ital. Biol.* **105**, 441 (1967); D. Ruggiero, R. R. Batton III, A. Jayaraman, M. B. Carpenter, *J. Comp. Neurol.* **172**, 189 (1977).
14. Supported by NIH grants NS12969 and DA01772.

25 July 1977; revised 25 October 1977