strate (Ventrex Laboratories) and the presence of the enzyme confirmed by inhibition with the converting enzyme inhibitor SQ 14,225 (Table 1).

Thus these cells contain all of the components of the complete renin-angiotensin system. Renin appears to be present in an inactive form. Although some investigators equate trypsin-activated renin with prorenin (*4*), the possibility that it represents renin bound to a protein inhibitor (*18*) cannot be excluded entirely. Inactive renin in plasma can be activated in vitro by exposure to an acid *p*H or to a variety of proteolytic enzymes including trypsin, kallikrein, and nerve growth factor (*19*). However, it is not established that this activation occurs in vivo. Similarly, the nature of this inactive renin and its endogenous mechanism of activation remains unknown. NG108-15 cells, a line of relatively homogeneous cells that express many properties of differentiated neurons, may prove useful in studies of the control of synthesis, secretion, and mechanism of action of renin and angiotensin within the nervous system.

The widespread presence of all components of the renin-angiotensin system in brain suggests that the role of this system may encompass more than blood pressure and volume regulation. The potential linkage of renin and angiotensin to processes of neuronal excitation (*20*) remains unexplored. It is possible that the entire renin-angiotensin cascade can be completed within a single nerve cell, and that control of the activation of intracellular renin might be a regulatory step in this pathway.

MARK C. FISHMAN*
Laboratory of Developmental Biology, National Institutes of Health, Bethesda, Maryland 20205
EARL A. ZIMMERMAN
Department of Neurology, College of Physicians and Surgeons, Columbia University, New York 10032
EVE E. SLATER
Medical Services, Massachusetts General Hospital, Boston, Massachusetts 02114

References and Notes

1. I. H. Page and J. W. McCubbin, *Renal Hypertension* (Yearbook, Chicago, 1969); R. Tigerstedt and P. G. Bergman, *Skand. Arch. Physiol.* **8**, 223 (1898).
2. M. I. Phillips, J. Weyhenmeyer, D. Felix, D. Ganten, W. Hoffman, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 2260 (1979); I. Reid, *ibid.*, p. 2225; M. I. Phillips, *Neuroendocrinology* **25**, 354 (1978); D. Ganten and G. Speck, *Biochem. Pharmacol.* **27**, 2379 (1978); T. Inagami, S. Hirose, H. Yokosawa, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 636 (Abstr.) (1979).
3. S. Hirose, H. Yokosawa, T. Inagami, *Nature (London)* **274**, 392 (1978).
4. _____, R. Workman, *Brain Res.* **191**, 489 (1980).
5. E. E. Slater, R. Defendini, E. A. Zimmerman, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5438 (1980).
6. B. Anderson and O. Westbye, *Life Sci.* **9**, 601 (1970).
7. T. Amano, E. Richelson, M. Nirenberg, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 258 (1972); P. Nelson, C. Christian, M. Nirenberg, *ibid.* **73**, 123 (1976).
8. S. Cohen, J. Taylor, K. Murakami, A. Michelakis, T. Inagami, *Biochemistry* **11**, 4286 (1972).
9. E. E. Slater, *Methods Enzymol.* **34**, 427 (1981).
10. A. Michelakis, H. Yoshida, J. Menzie, K. Murakami, T. Inagami, *Endocrinology* **94**, 1101 (1974).
11. K. Poulsen and J. Jorgensen, *J. Clin. Endocrinol. Metab.* **39**, 816 (1974).
12. J. Nussberger, R. Re, G. Matsueda, E. Haber, in preparation.
13. M. M. Kilcoyne, D. L. Hoffman, E. A. Zimmerman, *Clin. Sci.* **59**, 57S (1980).
14. L. A. Sternberger, *Immunocytochemistry* (Prentice-Hall, Englewood Cliffs, N.J., 1974).
15. Serum-free medium consisted of Ham's F12 medium supplemented with insulin (5 μg/ml), transferrin (100 μg/ml), putrescine (100 μ*M*), selenium (30 n*M*), and progesterone (20 n*M*) [J. E. Bottenstein and G. H. Sato, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 514 (1979)]. For these experiments cells were washed free of serum-containing medium twice over 30 minutes and then grown for 1 week with three changes of medium before analysis.
16. J. Minna. P. Nelson, J. Peacock, D. Glazer, M. Nirenberg, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 234 (1971); N. W. Seeds, A. G. Gilman, T. Amano, M. Nirenberg, *ibid.* **66**, 160 (1970).
17. B. Hamprecht, T. Amano, M. Nirenberg, unpublished data; A. Chalazonitis, J. Minna, M. Nirenberg, *Exp. Cell Res.* **105**, 269 (1977).
18. K. Poulsen, J. Kroll, A. H. Neilson, J. Jensenius, C. Malling, *Biochim. Biophys. Acta* **577**, 1 (1979).
19. J. Sealey, S. Atlas, J. Laragh, N. Oza, J. Ryan, *Nature (London)* **275**, 144 (1978); N. Yokosawa, N. Takahashi, T. Inagami, D. Page, *Biochim. Biophys. Acta* **569**, 211 (1976); B. Morris and D. Catanzaro, *IRCS Med. Sci. Physiol.* **8**, 433 (1980); B. J. Leckie and N. K. McGhee, *Nature (London)* **288**, 702 (1980).
20. M. C. Fishman, *Nature (London)* **260**, 542 (1976).
21. R. M. Cooper, G. E. Murray, D. H. Osmond, *Circ. Res.* **40**, I-171 (1977).
22. We thank P. G. Nelson and M. Nirenberg for helpful suggestions, M. Nirenberg for the NG108-15 cells, M. Karnovsky for the BHK cells, J. Nussberger for the angiotensin II antiserum, Squibb for the SQ 14,225, and S. Rosario, E. Piel, and H. V. Strout, Jr., for expert assistance. Supported in part by grants HL-21247 and HL-24105 from the National Institutes of Health. E.E.S. is an Established Investigator of the American Heart Association.
* Present address: Department of Medicine, Massachusetts General Hospital, 32 Fruit Street, Boston 02114.

1 May 1981; revised 12 August 1981

Both μ and δ Opiate Receptors Exist on the Same Neuron

Abstract. *Low concentrations of the relatively selective opiate receptor agonists dihydromorphine and normorphine (μ receptor agonists) and D-Ala²-D-Leu⁵-enkephalin (a δ receptor agonist) were applied to single enteric neurons while the frequency of action potential firing was recorded. Most neurons that were inhibited by the μ agonists were also inhibited by the δ agonist, but the two receptors could be distinguished by the higher concentration of naloxone required to antagonize the δ agonist. The results indicate that enteric neurons bear both μ and δ receptors and that cell firing is inhibited if either receptor type is activated.*

Plant opiates, synthetic opiate-like drugs, the naturally occurring opioid peptide enkephalin, and many enkephalin analogs all bind to a limited number of sites on neuronal membranes (*1–3*). Studies of the displacement of labeled ligands by unlabeled ligands (*3, 4*) and experiments in which binding site alkylation is prevented by prior exposure to an unlabeled ligand (*5, 6*) have provided convincing evidence that two binding sites can be distinguished. Pharmacological experiments also indicate two distinct sites, termed μ and δ receptors (*3*). The existence of two types of opiate receptors raises the question of whether they are borne by discrete neuronal populations and whether the functional con-

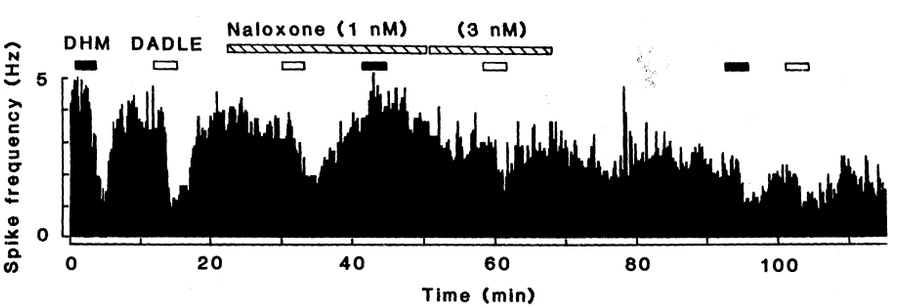

Fig. 1. Inhibition of neuronal firing by DHM (10 n*M*) and DADLE (1 n*M*). Binding studies carried out on membrane homogenates in tris buffer indicate that the ligands bind to distinct sites at these concentrations (*8*). Both agonists caused approximately equal but submaximal depression of firing. Naloxone (1 n*M*) completely prevented the effect of DHM but had little effect on the inhibition due to DADLE. A slightly higher concentration of naloxone (3 n*M*) largely prevented the effect of DADLE. Both agonists again inhibited cell firing after washout of the naloxone. The control firing rate of this neuron slowly declined during the 2-hour recording period.

0036-8075/81/1120-0923$01.00/0

Table 1. Number (and percentage) of myenteric neurons in which firing was inhibited by DMH or DADLE or both.

Treatment	Inhibition by DHM only	Inhibition by DADLE only	Inhibition by DHM and DADLE	No inhibition
DHM (10 n*M*) plus DADLE (1 n*M*)	25 (19)	5 (4)	39 (30)	60 (47)
DHM (30 n*M*) plus DADLE (10 n*M*)	2 (10)	3 (16)	11 (58)	3 (16)

sequences of their selective occupation are the same. However, it is difficult to examine this in the central nervous system in vivo because the concentrations of the selective ligands must be precisely known. The myenteric plexus of the guinea pig ileum contains binding sites for both the μ ligand dihydromorphine (DHM) and the δ ligand D-Ala^2-D-Leu^5-enkephalin (DADLE) (*7, 8*). We examined the effects of low concentrations of these substances on action potential discharge of single myenteric neurons in vitro and attempted to differentiate further between the two sites by applying naloxone.

The preparation was perfused at 37°C with a physiological saline solution and the action potentials of single myenteric neurons were recorded with glass suction electrodes (*9*). Drugs were applied by changing the superfusing solution to one which differed only in its content of the drugs. Both DHM and DADLE were applied to the same neurons. In the majority of experiments (129 neurons), 10 n*M* DHM and 1 n*M* DADLE were applied. It was considered that at these concentrations the agonists might act more or less selectively on μ and δ receptors (*8*). Firing of 47 percent of the 129 neurons was not affected by either ligand and firing of 30 percent was inhibited by both (Table 1). The degree of inhibition caused by DHM was approximately the same as that caused by DADLE (Fig. 1). Twenty-five neurons were inhibited by DHM but not by DADLE, whereas the firing of only five cells were inhibited by DADLE but not by DHM. The relatively small number of cells affected only by DADLE may have resulted from the low concentration used, because a concentration ten times higher selectively inhibited 16 percent of the cells (Table 1). Increasing the concentrations of DHM and DADLE increased the proportion of cells inhibited by both ligands (Table 1), perhaps because they no longer acted selectively on their receptors.

More naloxone is required to displace tritiated DADLE than to displace tritiated DHM from their binding sites in guinea pig ileum and cow brain (*8*); median inhibitory concentrations are 13.5 and 18.5 n*M* naloxone, respectively, for tritiated DADLE and 2.3 and 3.3 n*M* naloxone, respectively, for tritiated DHM. We therefore exposed cells inhibited by both agonists to a low concentration of naloxone (1 n*M*). This prevented the action of DHM without changing the inhibition induced by DADLE (Fig. 1). Since the initial degree of inhibition caused by 10 n*M* DHM was about the same as that caused by 1 n*M* DADLE, it appears that the two receptors are distinct. This effect of naloxone was reversed after the tissue was washed for several minutes (Fig. 1). In other experiments, increasing the concentration of naloxone to 10 n*M* resulted in antagonism of the effects of DHM and DADLE.

Whereas binding experiments show distinct μ and δ sites in the guinea pig myenteric plexus (*8*), pharmacological studies on the electrically induced, nerve-mediated contractile response of the longitudinal muscle have not detected a δ receptor in this tissue (*3, 8, 10*). Our results are direct evidence for the existence of a δ receptor and show that its occupation by an agonist has an effect—inhibition of cell firing—that is the same as the effect of a μ receptor agonist acting on a μ receptor on the same neuron. Important differences in the physiological consequences of μ and δ receptor agonists may exist which are beyond the resolution of our extracellular recording technique, but one common result of both is a depression of neuronal excitability.

TERRY M. EGAN
R. ALAN NORTH
Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge 02139

References and Notes

1. C. B. Pert and S. H. Snyder, *Science* **179**, 1011 (1973).
2. E. J. Simon, J. M. Hiller, I. Edelman, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1947 (1973).
3. J. A. H. Lord, A. A. Waterfield, J. Hughes, H. W. Kosterlitz, *Nature (London)* **267**, 495 (1977).
4. K.-J. Chang, B. R. Cooper, E. Hazum, P. Cuatrecasas, *Mol. Pharmacol.* **16**, 91 (1979).
5. L. E. Robson and H. W. Kosterlitz, *Proc. R. Soc. London Ser. B* **205**, 425 (1979).
6. J. R. Smith and E. J. Simon, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 281 (1980).
7. F. M. Leslie and H. W. Kosterlitz, *Eur. J. Pharmacol.* **56**, 379 (1979).
8. The binding of tritiated ligands to homogenates of myenteric plexus has been studied by F. M. Leslie, C. Chavkin, and B. M. Cox [*J. Pharmacol. Exp. Ther.* **214**, 395 (1980)]. Median inhibitory concentrations for displacement of tritiated DHM by DHM and DADLE were 1.94 and 15.6 n*M*, respectively; concentrations for displacement of tritiated DADLE by DHM and DADLE were 39.7 and 1.90 n*M*. It is difficult to apply these values to the present study because of the possibility of spare receptors and because the binding experiments were done at 23°C on homogenized membranes in the absence of sodium ions.
9. R. A. North and J. T. Williams, *Eur. J. Pharmacol.* **45**, 23 (1977).
10. This is probably because DADLE binds to μ and δ receptors when applied in a concentration sufficiently high to cause detectable inhibition of the twitch response; 1 n*M* DADLE does not cause detectable inhibition of the twitch response.
11. This work was supported by PHS grants DA01730, NS06672, and RCDA DA00068.

15 May 1981; revised 10 August 1981

Efferents to the Retina Have Multiple Sources in Teleost Fish

Abstract. *Multiple efferent systems project to the retina in three species of teleost fish investigated with the horseradish peroxidase technique. These animals are the first vertebrates shown to have more than one central nervous system structure projecting to the retina. The connections discovered may reflect a primitive organization of retina-brain interconnections.*

One of the questions of neurobiology is the significance of interspecific variability in brain organization. Certain cell aggregations present in some species are absent in others. An example of such variability is the absence or presence of efferent systems projecting to the retina. The most celebrated system of this kind is found in birds, in which a large, conspicuous cell group in the caudal mesencephalon projects to the contralateral eye (*1*). A comparable cell group has been observed in some reptiles (*2–4*), but has not been identified in other vertebrate classes (*5, 6*). Nevertheless, it has been argued that the cell group may exist in other vertebrates but that it lacks a projection to the retina (*7*). Optic nerve efferents have been described in mammals (*8*), although the sources of such fibers remain unknown (*6*). In reptiles a cell group in the ventral thalamus was recently identified with the horseradish peroxidase (HRP) technique. In the snake it is called the nucleus of the ventral supraoptic commissure (*4*) and in the lizard it is called the centrifugal optic thalamic nucleus (*9*). It is located near

0036-8075/81/1120-0924$01.00/0