warm water (*16, 17*), but island wind data suggest the rather abrupt appearance of remote westerlies prior to the main period of central Pacific equatorial warming for the post-1950 ENSO events (*18*). Certainly in 1982 the June westerlies appeared prior to the appearance of warm ocean surface water in the eastern central Pacific. The mechanisms of coupling between the ocean and atmosphere remain unclear.

The 1982 ENSO event in the ocean was unlike the composite post-1950 ENSO events in many ways (*19*), but each major event has included a period of at least weak central Pacific westerlies on the equator. There are indications that anomalous southerly flow between 150°E and 170°E preceded the main appearance of westerlies on the equator in the three major post-1950 events. However, the surface wind data for earlier events are very limited; 1982 may have been a special event. In any case, the southerly jet was a prominent feature of the low-frequency wind field of 1982 that appeared prior to the onset of both equatorial westerly winds west of the date line and significant ocean surface warming to the east of the date line, and deserves further study.

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References and Notes

1. S. G. H. Philander, *Nature (London)* **302**, 295 (1983).
2. "Anomalous" is used here to mean the departure from climatological conditions.
3. *Trop. Ocean-Atmos. Newsl. 16* (1983) (available from the Joint Institute for the Study of the Atmosphere and Ocean, University of Washington, Seattle).
4. *Trop. Ocean-Atmos. Newsl. 21* (1983).
5. D. E. Harrison and P. S. Schopf, *Mon. Weather Rev.*, in press.
6. T. P. Barnett, *ibid.* **111**, 756 (1983).
7. K. Wyrtki, *J. Phys. Oceanogr.* **5**, 572 (1975).
8. NOGAPS is a global weather prediction model consisting of independent global wind and mass analysis submodels, a global initialization which provides a dynamic balance of adjusted wind and mass fields, and a global primitive equation prediction model.
9. The climatology is based on the data set of merchant marine observations prepared by the National Climate Center. Data presented here are averaged over 2° areas of latitude and longitude, with no smoothing.
10. D. S. Luther and D. E. Harrison, *Mon. Weather Rev.*, in press.
11. E. M. Rasmusson and J. M. Wallace, *Science* **222**, 1195 (1983).
12. K. Wyrtki and G. Meyers's [Publication HIG-75-2 (Hawaii Institute of Geophysics, Honolulu, 1975)] fields of bimonthly average winds from ship reports suggest strongly anomalous southerly flow in the region of interest in September through October 1957, July through August 1965, and May through June 1972. Westerlies were observed on the equator in the following bimonthly period in each case.
13. H. Van Loon, "The southern oscillation, part III, Associations with the trades and with the trough in the westerlies of the South Pacific Ocean" (preprint).
14. E. M. Rasmusson and T. H. Carpenter, *Mon. Weather Rev.* **110**, 354 (1982).
15. P. A. Arkin *et al.*, *1982–1983 El Niño–Southern Oscillation Event Quick Look Atlas* (NOAA Climate Analysis Center, Washington, D.C., 1983).
16. A. E. Gill, *Q. J. R. Meteorol. Soc.* **106**, 447 (1980).
17. S. E. Zebiak, *J. Atmos. Sci.* **39**, 2017 (1982).
18. D. S. Luther, D. E. Harrison, R. A. Knox, *Science* **222**, 327 (1983).
19. M. A. Cane, *ibid.*, p. 1189.

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Impulse Activity Differentially Regulates [Leu]Enkephalin and Catecholamine Characters in the Adrenal Medulla

Abstract. *Regulation of the putative peptide neurohumour [Leu]enkephalin and the catecholaminergic enzymes tyrosine hydroxylase and phenylethanolamine-N-methyl-transferase was examined in the rat adrenal medulla in vivo and in vitro. Surgical denervation of the adrenal gland or pharmacologic blockade of synaptic transmission, treatments known to decrease catecholamine traits, increased [Leu]enkephalin content. Medullas explanted to culture exhibited a 50-fold rise in [Leu]enkephalin in 4 days, whereas tyrosine hydroxylase remained constant, and phenylethanolamine-N-methyltransferase decreased to a new baseline level. Veratridine-induced depolarization prevented the accumulation of [Leu]enkephalin, an effect that was blocked by tetrodotoxin, which antagonizes transmembrane Na^+ influx. These studies suggest that enkephalinergic and catecholamine characters are differentially regulated by impulse activity and depolarization in the adrenal medulla.*

Although the sympathoadrenal axis has long been known to play a role in adaptation to the environment, the complexity of molecular mechanisms underlying these responses has been appreciated only recently. Sympathoadrenal catecholamines integrate multisystem responses to stress, for example, but the function of adrenal opiate peptides is only now emerging. Nevertheless, opiate peptides and catecholamines already seem to participate in physiologic responses to hypotensive shock (*1*) and hypoxemia (*2*), and the peptides seem to mediate stress-induced analgesia (*3*). How does the adrenal medulla coordinate metabolism of these physiologically critical, diverse neurohormones?

Answers to this question may simultaneously elucidate new areas of physiologic regulation and define mechanisms governing neuroendocrine phenotypic plasticity. Neural crest derivatives, such as the adrenal medulla, are useful, simple model systems for studying cellular

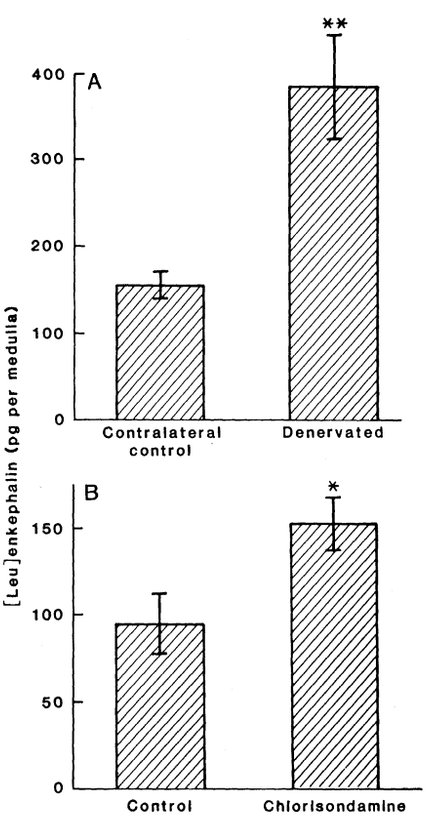


Fig. 1. Effects of decreased impulse activity on adrenal [Leu]enkephalin content. (A) Seven adult rats weighing 350 g were subjected to unilateral, surgical denervation of the adrenal gland. Denervated, contralateral control, and sham-operated adrenal glands were examined 4 days later for [Leu]enkephalin immunoreactivity (*11*). There was no significant differences in [Leu]enkephalin content between sham-operated and contralateral control adrenals. (B) Seven rats were treated with chlorisondamine (5 mg/kg, injected subcutaneously every 12 hours for 7 days). Adrenals were examined for [Leu]enkephalin content 12 hours after the last dose. Values for [Leu]enkaphalin are expressed as means ± standard errors. *Differs from control at $P < 0.05$. **Differs from control at $P < 0.01$ (two-tailed t-test).

expression of specific gene products like enzymes for catecholamines (*4*). Enkephalins and catecholamines are found together in adrenomedullary cells (*5–7*) and are released together by a number of stimuli (*6, 8*). Transsynaptic factors regulate enkephalin concentrations (*5, 9, 10*): adrenal denervation, or blockade of transsynaptic impulse flow, markedly increases enkephalin immunocytochemical reactivity in the rat adrenal medulla in vivo. To our knowledge, however, no studies have simultaneously analyzed catecholamine and enkephalin phenotypic plasticity in a parallel examination of the medulla in vivo and in vitro.

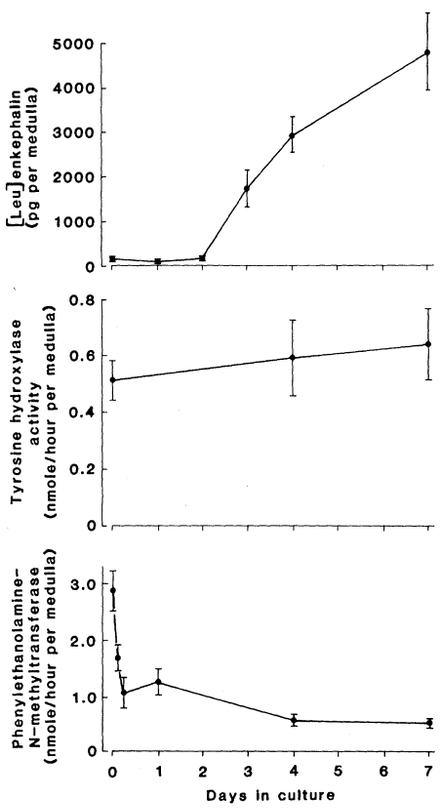


Fig. 2. Enkephalin and catecholaminergic enzymes in culture. Medullas, dissected free of surrounding cortex, were placed on filter-paper rafts in RPMI medium 1640 supplemented with 10 percent horse serum, 5 percent fetal calf serum, dexamethasone ($10^{-5}M$), penicillin (50 units per milliliter), and streptomycin (50 g/ml). Cultures were maintained at 37°C in an atmosphere of 95 percent air and 5 percent CO_2 at nearly 100 percent relative humidity. Medullas were examined after various times for [Leu]enkephalin content (picograms per medulla), TH activity (nanomoles per medulla per hour) (*11*), and PNMT activity (nanomoles per medulla per hour) (*13*). [Leu]enkephalin exhibited a significant increase, compared to zero time on and after day 3 ($P < 0.001$ based on individual comparisons by two-tailed *t*-test). Tyrosine hydroxylase activity did not significantly change, while PNMT activity was significantly lower than zero time by 2 and 4 hours after explantation and at each day thereafter ($P < 0.01$ and $P < 0.001$ based on individual comparisons by two-tailed *t*-test). Values are expressed as in Fig. 1 for seven medullas.

We have begun to characterize the roles of transsynaptic stimulation and depolarization in the regulation of adrenomedullary catecholaminergic and peptidergic traits. We used [Leu]enkephalin immunoreactivity to monitor peptidergic neurotransmitter content (*11*), and the enzymes tyrosine hydroxylase (TH) (*12*) and phenylethanolamine-*N*-methyltransferase (PNMT) (*13*) as indices of catecholamine regulation and phenotype. Tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, was used as an index of catecholamine metabolism as a whole, whereas PNMT, which synthesizes epinephrine, was used to assess adrenergic metabolism specifically.

To define the effects of transsynaptic stimulation in vivo, seven rats were subjected to unilateral surgical denervation of the adrenal gland or sham surgery. Transection of the splanchnic nerves innervating the adrenal resulted in a 145 percent increase in [Leu]enkephalin over control as determined by radioimmunoassay.

To determine whether the effects of denervation were due to interruption of impulse flow per se, rats were treated with chlorisondamine, a long-acting, nicotinic receptor antagonist. Treatment mimicked the effects of surgical denervation, significantly increasing medullary [Leu]enkephalin, supporting the contention that cholinergic, nicotinic stimulation normally decreases [Leu]enkephalin (Fig. 1).

To begin characterizing the molecular mechanisms regulating peptidergic and catecholaminergic traits, medulla were grown as explants in organ culture. The (denervated) explants exhibited a 50-fold rise in [Leu]enkephalin within 4 days in culture after an initial 2-day plateau, and continued rising through 1 week, the longest time examined (Fig. 2).

To determine whether catecholaminergic characters are similarly regulated, TH, which is present in all medullary chromaffin cells, was also examined. In contrast to [Leu]enkephalin, TH activity did not change during the entire culture period (Fig. 2). In addition PNMT, which is found only in the epinephrine-containing medullary cells, not only failed to increase in culture, but its activity decreased by approximately 60 percent during the first 4 hours in culture, maintaining stability thereafter (Fig. 2). The relative constancy of catecholamine traits could also be appreciated qualitatively at the cytochemical level; formaldehyde-induced catecholamine fluorescence and TH immunocytochemical reactivity, for example, were unchanged after 4 days in culture. Consequently, peptidergic and catecholaminergic regulation differ in medullary explants.

Explants were cultured in the presence of depolarizing agents to analyze mechanisms underlying transsynaptic regulation. Depolarizing concentrations of K^+ (*14*) prevented the accumulation of [Leu]enkephalin after explantation, thereby paralleling effects of transsynaptic stimulation in vivo (Fig. 3). Moreover, the depolarizing agent veratridine, which increases transmembrane Na^+ influx (*15*), also prevented the normal accumulation of [Leu]enkephalin (Fig. 4). Finally, tetrodotoxin, which antagonizes the effects of veratridine on Na^+ channels (*16*), blocked the effects of veratridine on [Leu]enkephalin (Fig. 4). We do not yet know whether the increase in

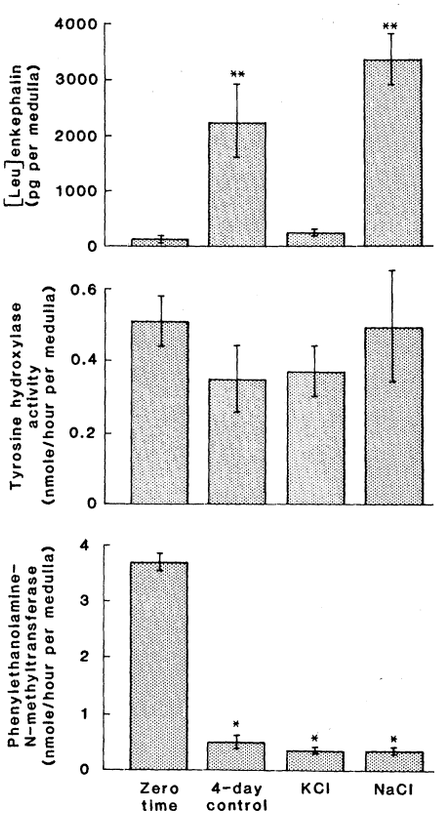
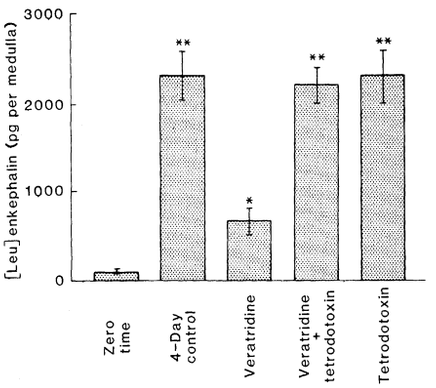


Fig. 3. Effects of membrane depolarization. Groups of seven medullas were cultured in the presence of KCl or NaCl (55 m*M*) added on day 3. Medullas were examined for [Leu]enkephalin content, TH, and PNMT activities on day 4. Potassium-induced depolarization prevented the accumulation of [Leu]enkephalin, whereas TH and PNMT activities were not significantly altered. Values are expressed as in Fig. 1. *Differs from zero time at $P < 0.001$; **Differs from zero time and KCl at $P < 0.001$ (one-way analysis of variance/Newman-Keuls and Kruskal-Wallis tests).

Fig. 4. Effects of veratridine. Groups of seven medullas were cultured in the presence of veratridine ($10^{-5}M$), tetrodotoxin ($10^{-7}M$), or both, added on day 3. Medullas were examined for [Leu]enkaphalin content on day 4. Veratridine-induced depolarization prevented the accumulation of [Leu]enkephalin. Tetrodotoxin blocked the effects of veratridine, while having no effect alone. Values are expressed as in Fig. 1. *Differs from zero time at $P < 0.05$. **Differs from zero time and veratridine at $P < 0.001$ (one-way analysis of variance and Newman-Keuls and Kruskal-Wallis tests).



[Leu]enkephalin after decreased transsynaptic activity in vivo or in vitro reflects increased synthesis, decreased release, decreased catabolism, or a combination of these processes. In contrast, however, depolarization did not significantly alter TH or PNMT activities (Fig. 3).

Our studies indicate marked differences in the regulation of [Leu]enkephalin and catecholaminergic characters in the adrenal medulla. Transsynaptic stimulation, which biochemically induces adrenomedullary TH and PNMT (*17*), dramatically decreases [Leu]enkephalin content. Reduction in peptide content is mediated by transmembrane Na^+ influx (Fig. 4), which mediates TH induction in sympathetic neurons in vitro (*18*). Consequently, the same or similar molecular events, depolarization and Na^+ influx, elicit opposite changes in peptidergic and catecholaminergic characters, which are found in the same medullary chromaffin cells. Although abundant evidence indicates that enkephalins and CA's, critical physiologic neurohormones, are co-localized and co-released by medullary cells, intracellular processing is distinct. Our observations suggest that diverse physiologic effectors, elaborated by the same cells, may be independently expressed and regulated. This study complements recent work from our laboratory, indicating that the peptides substance P and somatostatin are expressed and regulated differently from catecholamines in sympathetic neurons (*19*). Therefore, neuropeptides and "classical" neurohormones, such as catecholamines, may be differentially regulated in a wide variety of cell types.

In a somewhat different context, the independent regulation of sympathoadrenal neuropeptides and enzymes for catecholamines implies that physiological responses to stress (increased transsynaptic activity) may be analyzed in discrete molecular terms. It may be possible to identify metabolic regulatory and effector molecular mechanisms differentially elicited by variation in the local environment. Consequently, new therapeutic approaches to shock, hypoxemia, and stress-induced analgesia, for example, may evolve through the use of agents which differentially alter peptidergic or catecholaminergic metabolism.

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References and Notes

1. J. W. Holaday and A. I. Faden, *Nature (London)* **275**, 450 (1978); A. I. Faden and J. W. Holaday, *Science* **205**, 317 (1979); A. I. Faden *et al.*, *ibid.* **211**, 210 (1981).
2. M. Pokorski and S. Lahiri, *J. Appl. Physiol.* **51**, 1533 (1981); E. F. LaGamma *et al.*, *Am. J. Obstet. Gynecol.* **143**, 933 (1982).
3. J. W. Lewis, M. G. Tordoff, J. E. Sherman, J. C. Liebeskind, *Science* **217**, 557 (1982).
4. I. B. Black, *ibid.* **215**, 1198 (1982); N. M. Le-Douarin, *Nature (London)* **286**, 663 (1980); H. Thoenen, *Life Sci.* **14**, 223 (1974).
5. M. Schultzberg *et al.*, *Neuroscience* **3**, 1169 (1978).
6. O. H. Viveros, E. J. Diliberto, E. Hazum, K.-J. Chang, *Mol. Pharmacol.* **16**, 1101 (1979).
7. R. V. Lewis *et al.*, *Biochem. Biophys. Res. Commun.* **89**, 822 (1979); T. D. Hexum, H.-Y. T. Yang, E. Costa, *Life Sci.* **27**, 1211 (1980).
8. B. G. Livett *et al.*, *Nature (London)* **289**, 317 (1981).
9. R. V. Lewis *et al.*, *J. Neurosci.* **1**, 80 (1981).
10. M. C. Bohn, J. A. Kessler, L. Golightly, I. B. Black, *Cell Tissue Res.* **231**, 469 (1983).
11. Whole adrenal glands were removed, homogenized in 0.1*N* HCl and centrifuged at 50,000*g* for 20 minutes. The supernatant was separated, a portion was lyophilized, and duplicate samples were run by radioimmunoassay in *p*H 7.4 phosphate buffer [C. Gros *et al.*, *J. Neurochem* **41**, 29 (1978)]. Sensitivity for [Leu]enkephalin content was < 3 pg, with cross-reactivity of less than 3 percent for [Met]enkephalin. Additional antibody characteristics have been published (*10*).
12. I. B. Black, *Brain Res.* **96**, 170 (1975).
13. M. C. Bohn, M. Goldstein, I. B. Black, *Dev. Biol.* **89**, 299 (1982).
14. R. Nishi and D. K. Berg, *ibid.* **87**, 301 (1981).
15. W. Ulbricht, *Ergeb. Physiol. Biol. Chem. Exp. Pharmakol.* **61**, 18 (1969).
16. W. A. Caterall and M. Nirenberg, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3759 (1973).
17. H. Thoenen, R. A. Mueller, J. Axelrod, *Biochem. Pharmacol.* **19**, 669 (1970); *J. Pharmacol. Exp. Ther.* **169**, 249 (1969); *Nature (London)* **221**, 1264 (1969).
18. H. Bonisch, U. Otten, H. Theonen, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **313**, 199 (1980).
19. J. A. Kessler, J. E. Adler, M. Bohn, I. B. Black, *Science* **214**, 335 (1981); J. A. Kessler and I. B. Black, *Brain Res.* **234**, 182 (1982); J. A. Kessler, J. E. Adler, W. O. Bell, I. B. Black, *Neuroscience* **9**, 309 (1983).
20. Antibody to [Leu]enkephalin was provided by J. A. Kessler. We thank D. Straka, E. Grossman, B. Mayer, and T. Markey for technical assistance. E.F.L. is recipient of Clinical Investigator Award H.L 00756. J.E.A. is recipient of a John R. Whittier Fellowship, from the Committee to Combat Huntington's Disease. Supported by NIH grants HD12108 and NS10259 and aided by grants from the March of Dimes Birth Defects Foundation and the Cerebral Palsy Association.

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Chromosomal Location of Human Metallothionein Genes: Implications for Menkes' Disease

Abstract. *Human metallothioneins are encoded by a complex multigene family. The chromosomal location of these genes has been determined by gel transfer hybridization analysis of the DNA from human-rodent cell hybrids. Chromosome 16 contains a cluster of metallothionein sequences, including two functional metallothionein I genes and a functional metallothionein II gene. The remaining sequences, including a processed pseudogene, are dispersed to at least four other autosomes. The absence of metallothionein sequences from the X chromosome indicates that Menkes' disease, an X-linked disorder of copper metabolism, affects metallothionein expression by a* trans*-acting mechanism.*

Metallothioneins (MT's) are cysteine-rich, low molecular weight proteins that bind heavy metal ions including zinc, copper, cadmium, and mercury (*1*). They are believed to play a role in both heavy metal homeostasis and detoxification. All vertebrates examined synthesize two major isoforms, MT-I and MT-II, that have closely related but distinct amino acid sequences. In man, results obtained by protein sequencing suggest the presence of at least three different forms of MT-I. In addition, gel transfer hybridization analysis has demonstrated multiple MT-related sequences in the human genome (*2*). To further our understanding of the organization and evolution of this multigene family, we have performed chromosome mapping experiments on a series of human-rodent cell hybrids. We were particularly interested in determining whether any MT sequences are located on the X chromosome because of the evidence that Menkes' disease (*3*), an X-linked disorder of copper metabolism, alters either