generated by a decrease in resting $Na^+$ and $K^+$ conductance.

Alternative explanations are possible that are consistent with the demonstration of a reversal potential for the hyperpolarization. The NA effect could be to reduce the ongoing release of depolarizing transmitter substance (*25*). We have not, however, observed decreases in synaptically evoked potentials during NA application, except those explicable by the NA-induced hyperpolarization, so that such an effect seems unlikely. A third possibility is the generation of two effects by the NA, a hyperpolarization (for instance, by activation of an ion transport system) and a general decrease in membrane conductance. Such a combination would exhibit an apparent reversal potential during the intracellular injection of current. The value of this reversal level would occur when the product of the injected current and the change in cell input resistance is equal to the amplitude of the hyperpolarization.

The third possibility would be compatible with recent proposals that catecholamines may hyperpolarize frog sympathetic ganglion neurons (*26*), mammalian central neurons (*27*), or striated muscle (*28*) by stimulation of a $Na^+$-$K^+$ pump. In addition, association of pump activation with decreases in membrane conductance has been observed in striated muscle (*29*).

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### References and Notes

1. D. G. Amaral and H. M. Sinnamon, *Prog. Neurobiol.* **9**, 147 (1977).
2. J. W. Phillis, A. K. Tebecis, D. H. York, *Eur. J. Pharmacol.* **4**, 471 (1968).
3. G. R. Siggins, A. P. Oliver, B. J. Hoffer, F. E. Bloom, *Science* **171**, 192 (1971).
4. I. Engberg and K. C. Marshall, *Acta Physiol. Scand.* **83**, 142 (1971).
5. A. P. Oliver and M. Segal, *Soc. Neurosci. 4th Annu. Meet. Abstr.* (1974), p. 361.
6. L. Bonkowski and W. F. Dryden, *Neuropharmacology* **16**, 89 (1977).
7. F. F. Weight, in *The Neurosciences, Third Study Program*, F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, Mass., 1974), p. 929.
8. J. E. Brown, K. J. Mullen, G. Murray, *Science* **174**, 318 (1971).
9. F. F. Weight and A. Padjen, *Brain Res.* **55**, 219 (1973); *ibid.*, p. 225.
10. H. M. Gerschenfeld and D. Paupardin-Tritsch, *J. Physiol. (London)* **243**, 427 (1974); *ibid.*, p. 457.
11. C. C. Huang and A. S. Marrazzi, *Soc. Neurosci. 3rd Annu. Meet. Abstr.* (1973), p. 292.
12. J. C. Delaleu, *J. Exp. Biol.* **65**, 117 (1976).
13. I. Engberg, Y. Källström, K. C. Marshall, *Acta Physiol. Scand.* **84**, 4A (1972). The electrode attached to the multibarrelled assembly was first driven into the gray matter of the cord. After a stable penetration, the prealigned single electrode was lowered into the same region. With cells that were less than 1.5 mm below the (lateral) cord surface, penetration of the same cell was usually achieved by the second electrode.
14. A preliminary report of these findings has been given in I. Engberg and K. C. Marshall, *J. Gen. Physiol.* **61**, 261 (1973).
15. The NA was passed by using a positive current, from a 0.5*M* solution, *p*H 5. Current controls were either in the form of balancing opposite currents from another electrode of the assembly or of comparing NA effects with responses to equivalent or higher sodium currents passed from another electrode. Intracellular electrodes were usually filled with KCl, sometimes with citrate.
16. P. E. Voorhoeve, *Acta Physiol. Pharmacol. Neerl.* **9**, 1 (1960).
17. Electrical coupling between the screened intracellular current-passing and recording electrodes was often so small that it could be neglected. In other cases, the coupling was measurable by oscilloscopic examination of the rising and falling phases of short current pulses. The amplitude of the coupling was a linear function of the current amplitude, and was easily corrected by subtracting the appropriate value from the apparent potential displacement resulting from a given current pulse.
18. K. C. Marshall and I. Engberg, *Can. Physiol.* **4**, 188 (1973); I. Engberg and K. C. Marshall, *Neuroscience*, in press.
19. I. Engberg, J. A. Flatman, K. Kadzielawa, *Acta Physiol. Scand.* **96**, 137 (1976).
20. ———, *ibid.* **91**, 3A (1974).
21. K. C. Marshall and I. Engberg, *Proc. Can. Fed. Biol. Soc.* **16**, 33 (1973).
22. M. Segal and F. E. Bloom, *Brain Res.* **72**, 99 (1974).
23. G. R. Siggins, B. J. Hoffer, A. P. Oliver, F. E. Bloom, *Nature (London)* **233**, 482 (1971); B. J. Hoffer, G. R. Siggins, A. P. Oliver, F. E. Bloom, *J. Pharmacol. Exp. Ther.* **184**, 553 (1973).
24. B. J. Hoffer, G. R. Siggins, A. P. Oliver, F. E. Bloom, *Ann. N.Y. Acad. Sci.* **185**, 531 (1971).
25. H. M. Gerschenfeld, in *International Cell Biology, 1976-1977*, B. R. Brinkley and K. R. Porter, Eds. (Rockefeller Univ. Press, New York, 1977), p. 93.
26. K. Koketsu and M. Nakamura, *Jpn. J. Physiol.* **26**, 63 (1976).
27. J. W. Phillis, *Life Sci.* **15**, 213 (1974); G. G. Yarbrough, *Neuropharmacology* **15**, 335 (1976); U. Heinemann, H. D. Lux, K. J. Zander, in *Iontophoresis and Transmitter Mechanisms in the Mammalian Central Nervous System*, R. W. Ryall and J. S. Kelly, Eds. (Elsevier/North-Holland, Amsterdam, 1978), p. 419.
28. E. T. Hays, T. M. Dwyer, P. Horowicz, J. G. Swift, *Am. J. Physiol.* **227**, 1340 (1974); B. H. Bressler, J. W. Phillis, W. Kozachuk, *Eur. J. Pharmacol.* **33**, 201 (1975).
29. T. Clausen and J. A. Flatman, *J. Physiol. (London)* **270**, 383 (1977); D. Geduldig, *ibid.* **194**, 521 (1968).
30. We thank Hegström for technical assistance and G. W. Mainwood for critically reviewing the manuscript. The experiments described here were conducted at the Physiology Institute, University of Göteborg, Göteborg, Sweden.

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## Dual Mechanism Mediating Opiate Effects?

Jacquet has proposed (*1*) that morphine acts on two different receptors in the brain: a naloxone-sensitive endorphin receptor that mediates the analgesic and catatonic effects of morphine, and a naloxone-insensitive adrenocorticotropic hormone (ACTH) receptor that mediates the opiate abstinence syndrome and the excitatory effect of the drug. We see value in some aspects of this formulation but take exception to others.

Jacquet reports that the opioid peptides β-endorphin and Met-enkephalin (*2*) fail to mimic the behavioral excitation produced by morphine or ACTH. These peptides, according to Jacquet, should not produce opiate-like dependence. Earlier findings indicate that repeated injections of endorphins into the periaqueductal gray (PAG) result in physical dependence as manifested by the occurrence of withdrawal symptoms following naloxone injections or cessation of endorphin administration (*3*). Jacquet has attributed these findings to PAG damage caused by the large cannula (*4*) used to deliver endorphin. Indeed, we (*5*) observed explosive motor behavior (EMB) immediately after lesions to the PAG. In Wei and Loh's study (*3*), however, infusion of water to the PAG through identical large cannulas did not trigger EMB. Thus, the withdrawal signs observed by Wei and Loh (*3*) may be attributed to endorphins and not to procedural artifacts. Furthermore, the opioids levorphanol and etonitazene produce physical dependence in animals and in humans (*6*), but fail to precipitate EMB when injected intracerebrally in naloxone-treated or -naïve animals (*7*). These findings demonstrate that the ability of opioids to produce physical dependence is not conditional upon their ability to elicit behavioral excitation (that is, EMB).

We question the use of the label "ACTH receptor" to describe the site at which ACTH and opiates are producing EMB. Wei *et al.* (*8*) reported that intracerebral injections of thyrotrophin-releasing hormone in rats induce opiate withdrawal-like, wet-dog shakes. We observed (*9*) EMB after intraventricular injections of either lithium or various calcium chelators, the latter at molar doses below those effective in morphine EMB. Given that the class of substances capable of producing EMB may be fairly large, the designation ACTH receptor may be misleading. In fact, there is evidence that ACTH may act at the endorphin receptor. Some aspects of ACTH-induced behavioral excitation are modified by naloxone (*10*). ACTH has affinity for opiate receptors in vitro and it antagonizes morphine analgesia in animals (*11*). Thus, the excitatory effects of ACTH may not be independent of its action at the stereospecific endorphin receptor. While we agree that the mechanism subserving EMB may play a role in


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the behavior seen during opiate withdrawal, we cannot accept the notion, implied by Jacquet's formulation, that the only compounds that can induce opiate-like dependence are those that can also produce EMB, and that an ACTH receptor is critically involved in this effect.

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**References and Notes**

1. Y. F. Jacquet, *Science* **201**, 1032 (1978).
2. J. D. Belluzzi, N. Grant, V. Gassky, D. Sarantakis, C. D. Wise, L. Stein, *Nature (London)* **260**, 625 (1976); H. Loh, L. F. Tseng, E. Wei, C. H. Li, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2895 (1976).
3. E. Wei and H. Loh, *Science* **193**, 1262 (1976).
4. Wei and Loh, (*3*) have used cannulas with an outer diameter of 0.81 mm. Jacquet, in her study, used cannulas with an outer diameter of 0.30 mm.
5. R. Blair, J. Liran, H. Cytryniak, P. Shizgal, Z. Amit, *Neuropharmacology* **17**, 205 (1978).
6. H. Isbell and H. Fraser, *J. Pharmacol. Exp. Ther.* **107**, 524 (1953); A. Wikler, M. Martin, F. Fraser, R. Eades, *Psychopharmacologia* **5**, 55 (1963).
7. R. Blair, H. Cytryniak, P. Shizgal, Z. Amit, *Behav. Biol.* **24**, 24 (1978).
8. E. Wei, S. Sigel, H. Loh, E. L. Way, *Nature (London)* **253**, 739 (1975).
9. R. Blair, D. Sinyor, H. Cytryniak, L. Switzman, Z. Amit, paper presented at the American Psychological Association meeting, Toronto, 1978.
10. W. H. Gispen and V. M. Wiegant, *Neurosci. Lett.* **2**, 159 (1976).
11. E. Zimmerman and W. Krivoy, *Prog. Brain Res.* **39**, 383 (1973); L. Terenius, W. H. Gispen, D. DeWied, *Eur. J. Pharmacol.* **33**, 395 (1975).

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The objection of Amir *et al.* to Jacquet's formulation (*1*) of a dual mechanism mediating opiate effects, with the endorphin receptor mediating narcotic analgesia-catatonia, and the adrenocorticotropic hormone (ACTH) receptor mediating opiate excitation and abstinence behavior, appear to center on the ACTH receptor. Their criticisms of this latter mechanism fall into two categories:

1) The occurrence of explosive motor behavior (EMB) after periaqueductal gray (PAG) injections of $\beta$-endorphin (*2*), or intracerebroventricular (ICV) injections of compounds other than opiates or ACTH (lithium or calcium chelators), and the nonoccurrence of EMB after ICV injections of some opiates such as levorphanol and etonitazene.

2) Some in vitro (*3*) and in vivo (*4*, *5*) effects of ACTH that appear to be at variance with the view of a receptor other than the endorphin receptor that mediates some of morphine's actions.

I deal here with these points in this sequence.

1) To date, there is only one report (*2*) on the dependence liability of $\beta$-endorphin after direct, chronic administration to brain. (The direct route to brain is necessary since it is not yet established that this peptide can cross the blood-brain barrier as an intact peptide.) In this investigation, it is not clear whether the infused peptide was actually the intact, or an altered form of $\beta$-endorphin since the subcutaneously implanted osmotic minipump, maintained at body temperature, served as the reservoir for $\beta$-endorphin over the 70-hour infusion period. Moreover, considering the large size of the intracerebral cannula (outer diameter, 0.81 mm), it is probable that the site of infusion may have overlapped with the aqueduct or fourth ventricle, thereby allowing the injected peptide to diffuse to other central nervous system (CNS) sites through the ventricular fluid. These possible sources of error may explain why abstinence signs (abnormal posture, ear blanching, licking, and ptosis) occurred during the period of infusion, although the authors attribute the reason to be the short interval between surgery and experimentation (which may have been another source of error).

The argument concerning the occurrence or nonoccurrence of EMB following the ICV injection of various compounds fails to make an important distinction between ICV and PAG injections. The former is nonspecific with respect to site, and therefore mechanism of action (that is, the CNS site or sites which mediate the behavioral effects remain unidentified and can be any site adjacent to the ventricles), while the latter is specific, and more to the point, specific to the site where morphine exerts its effects of analgesia-catatonia and EMB. I have previously reported (*6*) that PAG injections of some opiates (methadone, levorphanol, etorphine) failed to result in EMB, and only high doses of these agents were able to achieve a mild degree of analgesia. This was seen as due to the high lipophilicity of these opiates which allowed rapid diffusion throughout the CNS. This diffusion, at the same time, activated those CNS sites which exerted an inhibitory influence on the excitatory action of the opiate at the ACTH receptor. In this way, some opiates with high dependence liability fail to cause EMB when injected into the PAG, while the low lipophilicity of morphine allows local effects at the PAG to be expressed.

2) That ACTH(1-24) at $10^{-6}M$ competitively displaced [$^3$H]dihydromorphine from binding sites in brain (*3*) is not incompatible with the view that morphine effects are mediated by two receptors, a stereospecific endorphin receptor, and a nonstereospecific ACTH receptor. Moreover, since ACTH has opposite effects from endorphin, it is not surprising that ACTH can antagonize morphine analgesia (mediated by the endorphin receptor) (*4*).

Excessive grooming induced by ICV injection of ACTH (*5*) may have been due to a degradation product of ACTH, since this peptide is known to undergo rapid degradation in brain. Naloxone antagonism of this effect may have been mediated by a nonopiate mechanism, since naloxone has been reported to antagonize the effects of nitrous oxide, $\gamma$-aminobutyric acid, acetylcholine, and so on.

In view of the finding (*8*) that both $\beta$-endorphin and ACTH are derived from the same "31 k" precursor, it is not unreasonable to assume that these two peptides have correlated functions in the regulation of behavior.

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**References and Notes**

1. Y. F. Jacquet, *Science* **201**, 1032 (1978).
2. E. Wei and H. H. Loh, *ibid.* **193**, 1262 (1976).
3. L. Terenius, W. H. Gispen, D. deWied, *Eur. J. Pharmacol.* **33**, 395 (1975).
4. E. Zimmerman and W. Krivoy, *Prog. Brain Res.* **39**, 383 (1973).
5. W. H. Gispen and V. M. Wiegant, *Neurosci. Lett.* **2**, 159 (1976).
6. Y. F. Jacquet and A. Lajtha, *Science* **185**, 1055 (1974).
7. Y. F. Jacquet, W. A. Klee, K. C. Rice, I. Iijima, J. Minamikawa, *ibid.* **198**, 842 (1977).
8. R. E. Mains, B. A. Eipper, N. Ling, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3014 (1977).

8 June 1979