References and Notes

1. E. B. Truitt and M. J. Walsh, in *The Biology of Alcoholism*, B. Kissin and H. Begleiter, Eds. (Plenum, New York, 1971), vol. 1, pp. 161-195.
2. L. D. MacLeod, *Q. J. Stud. Alcohol* **11**, 385 (1950).
3. S. G. Holtzman and F. H. Schneider, *Life Sci.* **14**, 1243 (1974).
4. H. Loomis, E. N. Harvey, G. Hobart, *J. Exp. Psychol.* **19**, 239 (1936); W. J. Horsey and K. Akert, *Q. J. Stud. Alcohol* **14**, 363 (1953); E. K. Sauerland and R. M. Harper, *Exp. Neurol.* **27**, 490 (1970); G. Dolce and H. Decker, *Res. Commun. Chem. Pathol. Pharmacol* **3**, 523 (1972); W. R. Klemm, *Nature (London)* **251**, 234 (1974).
5. R. A. Beck, C. C. Pfeiffer, V. Iliev, L. Goldstein, *Proc. Soc. Exp. Biol. Med.* **128**, 823 (1968).
6. H. Ogata, *Med. J. Shinshu Univ.* **9**, 171 (1964).
7. J. Akabane, *ibid.* **5**, 113 (1960).
8. M. H. Walsh, P. B. Hollander, E. B. Truitt, *J. Pharmacol. Exp. Ther.* **167**, 173 (1969).
9. E. Majchrowicz, B. L. Bercaw, W. M. Cole, D. H. Gregory, *Q. J. Stud. Alcohol* **28**, 213 (1967).
10. The cortical electrodes were fabricated from 30-gauge Formvar-insulated, nichrome wire. The two frontal electrodes were placed 1 mm anterior to bregma, and 2 mm on either side of the midline. The parietal electrodes were placed bilaterally 3 mm posterior to bregma and 3 mm from the midline.
11. Local anesthesia of wound margins was produced by means of lidocaine hydrochloride. Neuromuscular blockage was induced by Flaxedil (0.01 mg/kg).
12. Spontaneous EEG was recorded on a Grass model 78 polygraph with 7P511 amplifiers. Some data were stored on magnetic tape with an Amplex model PR 500 recorder-reproducer.
13. The EEG signals were monitored at three different passbands to facilitate visual detection of drug-induced changes. The three passbands used were 0.3 to 90 Hz, 10 to 90 Hz, and 0.3 to 30 Hz.
14. W. R. Klemm, *Animal Electroencephalography* (Academic Press, New York, 1969).
15. For each animal, heart rate was sampled at successive 10-second intervals. Sampling began 100 seconds before the beginning of injections or infusions and ended 150 seconds after the start of injections or the end of infusions. For each infusion or injection dose the mean heart rate for each 10-second interval was obtained by averaging across animals. The mean heart rate before injection for all animals at a given dose was determined by averaging the ten means obtained before injection (or before infusion). The maximum change in heart rate is expressed as a deviation from this mean heart rate before injection.
16. In all experiments, the order of administration of different acetaldehyde doses (and saline) was randomized. All infusions lasted 240 seconds.
17. C. J. P. Eriksson, H. W. Sippell, O. A. Forsander, in *The Role of Acetaldehyde in the Actions of Ethanol*, K. O. Lindros and C. J. P. Eriksson, Eds. (Kauppakirjapaino, Helsinki, 1975), pp. 9-18; G. Duritz and E. B. Truitt, *Q. J. Stud. Alcohol* **25**, 498 (1964). Blood samples were prepared for analysis by adding 100 μl of arterial blood to 100 μl of a solution containing $HClO_4$ (0.6*M*), thiourea (25 m*M*), and *t*-butanol (300 ng/μl) in a 12-ml rubber-capped serum vial. *t*-Butanol was included as an internal standard for ensuring uniformity of injections. A Hewlett-Packard model 5710A gas chromatograph with a 0.4 by 180 cm glass column packed with Chromosorb 101 (Johns-Manville) was used for separation (column temperature, 160°C; injection port 200°C; flame ionization detector 250°C; helium flow rate, 60 ml/min). The samples were equilibrated for at least 30 minutes at room temperature. Portions (500 μl) of headspace gas were injected with a (gastight) 1-ml disposable plastic syringe. Headspace of reference standards (100 ng/μl and 33 ng/μl) was injected repeatedly during each experiment. Injections were discounted if the *t*-butanol amplitude deviated by more than 10 percent from the mean value for the series. Reproducibility of the technique with replicates of standard test solutions was better than 93 percent.
18. H. W. Sippell and C. J. P. Eriksson, in *The Role of Acetaldehyde in the Actions of Ethanol*, K. O. Lindros and C. J. P. Eriksson, Eds. (Kauppakirjapaino, Helsinki, 1975), pp. 149-157.

19. We thank K. H. Dahm for technical advice and supervision regarding the gas chromatographic analyses.

12 September 1978

Stimulation of Human Periaqueductal Gray for Pain Relief Increases Immunoreactive β-Endorphin in Ventricular Fluid

Abstract. *Immunoreactive β-endorphin was measured in the ventricular fluid of six patients with chronic pain. Stimulation of the periaqueductal gray matter in three patients with pain of peripheral origin resulted in significant increases (50 to 300 percent) in the concentration of ventricular immunoreactive β-endorphin. In three other patients suffering deafferentation dysesthesia, stimulation of the posterior limb of the internal capsule did not alter the concentration of this peptide. These results provide evidence of the release of human immunoreactive β-endorphin in vivo and suggest that naloxone-reversible pain relief achieved by stimulation of the periaqueductal gray matter may be in part mediated by the activation of β-endorphin-rich diencephalic areas.*

Since the initial report by Reynolds (*1*) of analgesia being produced in the rat by stimulation of the periaqueductal gray matter, several workers have demonstrated the potent analgesic effects produced by electrical stimulation of discrete areas of the medial diencephalon and brainstem in the cat (*2*), monkey (*3*), and rat (*4*). Hosobuchi *et al.* (*5*) and Richardson and Akil (*6*) have reported that intractable clinical pain states in humans, in addition to normal pain perception, can be blocked by electrical stimulation of the periaqueductal and periventricular gray matter. This pain relief can be totally reversed by the specific opiate antagonist naloxone (*5*, *7*).

Intraventricular administration of human β-endorphin in humans produces a prolonged state of analgesia (*8*). The current study was undertaken to determine whether the analgesia produced by stimulation of the periaqueductal gray matter would be accompanied by increases in the β-endorphin concentrations in ventricular cerebrospinal fluid (CSF).

The specificity of the effect of stimulating the periaqueductal gray matter for the endorphin system was also investigated. For this purpose we selected two groups of patients: group 1, suffering from deafferentation pain; and group 2, whose pain relief is known to be obtained by stimulation of the periaqueductal gray matter or by opiates. In group 1, pain relief is obtained neither by opiates nor by periaqueductal gray matter stimulation, but it can be obtained by electrical stimulation of the internal capsule (*9*). Therefore, should our working hypothesis linking stimulation of periaqueductal gray matter with the endorphin system be correct, treatment of deafferentation pain by internal capsule stimulation should not affect β-endorphin concentrations in ventricular fluid.

Ventricular fluid was collected from six patients undergoing stereotactic implantation of brain electrodes for pain control (Table 1). The three patients in group 1, suffering from deafferentation pain, received electrode implantation in the posterior limb of the internal capsule contralateral to their pain (patients A, B, and C). The patients in group 2, with pain of peripheral origin, and received bilateral implantations of electrodes in the rostral portion of the periaqueductal gray matter (patients D, E, and F). A Leksell stereotactic frame was used on all six patients and the stereotactic coordinates were selected and calculated on the basis of Schaltenbrand and Bailey's human stereotactic atlas (*10*). Coordinates for the posterior limb of the internal capsule were: anteroposterior axis at the line intercepting the anterior and posterior commissure; and ventrodorsal axis 1 mm below the intercommissural line and 15 to 20 mm lateral from the midline of the brain, depending on the portion of the body for which relief of pain is sought, the upper limb being more medial than the lower limb in the "homuncular" representation of the internal capsule. For periaqueductal implantation, the area coordinates were at

Table 1. Clinical summary of the patients (*17*).

Patient	Age	Sex	Etiology of pain	Location of pain
A	47	M	Postcordotomy dysesthesia	Bilateral, lower extremities
B	54	M	Postcordotomy dysesthesia	Right lower extremities
C	63	M	Thalamic syndrome	Right arm and leg
D	51	M	Lumbosacroarachnoiditis	Low back and both legs
E	57	F	Carcinoma of rectum	Abdomen and perineum
F	66	F	Carcinoma of colon	Abdomen

0036-8075/79/0119-0279$00.50/0

Table 2. The concentration of immunoreactive β-endorphin in the ventricular fluid of six patients. Ventricular CSF samples were collected from a ventricular catheter inserted to perform intraoperative ventriculography. The tip of the catheter was usually located just at the foramen of Monro, but not in the third ventricle. Two milliliters of ventricular fluid were collected (i) at the onset of the surgery prior to ventriculography, insertion of electrode, and stimulation (baseline); (ii) at the end of the 15-minute stimulation period; (iii) 15 minutes after the cessation of stimulation; and (iv) 30 minutes after cessation. Further collection of the fluid would have prolonged the usual time required for the closure of the surgical wound; it was thus not attempted. Tubes containing the collected ventricular fluids were immediately immersed in boiling water for 10 minutes to halt possible peptide degradation, and then frozen. After lyophilization, samples were redissolved in one-fifth of their original volume with the following solution: 0.02*M* sodium phosphate buffer, *p*H 7.5, with 0.145*M* NaCl, 0.1 percent gelatin, 0.01 percent thimerosal, 0.01 percent crystalline bovine serum albumin, and 0.1 percent Triton X-100. Radioimmunoassays for β-endorphin were performed as described (*12*), in duplicate at three doses—that is, 40, 80, and 100 μl. The dose-response curves were parallel to the standard curves. Our radioimmunoassay for β-endorphin shows complete cross-reactivity with β-lipotropin. The amount of material obtained during the neurosurgical procedure was insufficient to characterize immunoreactive β-endorphin by gel filtration. We showed previously (*13*) that immunoreactive β-endorphin in the rat brain was mainly authentic β-endorphin.

Patient	β-Endorphin (pg/ml)			
	Baseline	End of stimulation period	15 minutes after cessation of stimulation	30 minutes after cessation of stimulation
A	180	190	190	170
B	140	140	140	*
C	160	160	*	*
D	170	230	270	*
E	210	650	720	640
F	200	1410	720	980

*Not collected.

the level of the posterior commissure on the anteroposterior axis and ventrodorsal axis, and 2 to 3 mm lateral from the lateral wall of the posterior third ventricle or aqueduct.

The electrodes were implanted stereotactically as described (*5*). Electrode location was verified by intraoperative x-ray. As soon as the electrodes were inserted, they were activated for 15 minutes to verify the efficacy of the relief of pain. Stimulation parameters were 3 to 10 V, 5- to 20-Hz biphasic wave for electrodes located in the central gray matter; this produced no accompanying sensation or discomfort (*5*). The stimulation of the posterior limb of the internal capsule is accompanied by paresthesia in the contralateral side of the body (*11*); parameters for this stimulation were 3 to 5 V, 50- to 75-Hz biphasic wave.

All six patients reported here exhibited significant or complete pain relief with either internal capsule or periaqueductal gray stimulation. Ventricular fluids were collected and radioimmunoassays for β-endorphin were performed as described (*12*).

Control concentrations of immunoreactive β-endorphin in the ventricular fluid of the patients ranged from 140 to 210 pg/ml. Stimulation of the internal capsule was not followed by any increase in immunoreactive β-endorphin above the control concentrations. There was, however, a two- to sevenfold increase in the concentration of immunoreactive β-endorphin of the three patients after stimulation of the periaqueductal area (Table 2).

In two of these patients (E and F), radioimmunoassays for leucine-5-enkephalin (Leu^5-enkephalin) were also performed. As previously described (*13*), this assay is highly specific for Leu^5-enkephalin because it does not react with α-, β-, γ-, or δ-endorphin but does show a 3 percent cross-reactivity with methionine-5-enkephalin. The sensitivity of this assay is such that it could have detected as little as 25 pg of Leu^5-enkephalin per milliliter of original ventricular fluid. No Leu^5-enkephalin immunoreactive material could be detected in the ventricular fluid of either patients E or F, before or after stimulation of the periaqueductal gray.

In the rat brain, fibers that are immunoreactive for β-endorphin are highly concentrated in the anterior periaqueductal gray matter. These fibers are part of the long projections of the β-endorphin-immunoreactive cell bodies located in the basal tuberal hypothalamus (*14*). Fibers of this pathway are somewhat dense around the wall of the third ventricle, especially in the anterior part of the ventricle. Furthermore, the anterior part of the rat hypothalamus exhibits the highest concentration of immunoreactive β-endorphin (*13*). It seems possible that the immunoreactive β-endorphin observed here in samples of CSF from the third ventricle could come from these β-endorphin-containing fibers packed in the anterior hypothalamus, and that electrical stimulation of the periaqueductal gray matter might induce antidromic stimulation of the anterior hypothalamic β-endorphin fibers and the subsequent release of β-endorphin into the third ventricle.

In the case of deafferentation dysesthesia, stimulation of the periaqueductal gray matter is ineffective in producing pain relief; opiates are also ineffective (*9*). In these cases, pain is relieved by long-term stimulation of the somatosensory system, the effect presumably being mediated by an activation of the inhibitory corticofugal fibers (*9*). This relief is not reversed by naloxone, nor is it accompanied by increased concentrations of immunoreactive β-endorphin in the CSF.

Naloxone has been shown to reverse the pain relief obtained from intraventricularly injected β-endorphin in humans (*8*), as well as from stimulation of periaqueductal gray matter. Therefore, the mechanisms underlying the pain relief obtained by these two methods may well be similar. The only other known endogenous opioid peptides in the periaqueductal gray area, the enkephalin pentapeptides, are known to produce only transient analgesia in experimental animals (*15*) and are therefore less likely to be the source of the longer-lasting analgesia observed in humans after stimulation of the periaqueductal gray matter. On the basis of data from an assay of the isotopically labeled receptors, Akil *et al.* (*16*) reported that stimulation of periaqueductal gray matter produced a moderate increase (50 percent) in an uncharacterized enkephalin-like compound in the third ventricle. In this study we found no increase of Leu^5-enkephalin immunoreactivity.

Our findings demonstrate that the pain relief produced by stimulation of periaqueductal gray matter has considerable anatomical and pharmacological specificity, inasmuch as stimulation of the internal capsule resulted in no changes in the concentration of immunoreactive β-endorphin in the CSF. However, we need to determine how much the "basal" levels measured here differ from levels in unstressed patients not undergoing neurosurgical procedures, and whether the site of the CSF collection is critical in measuring increments of change in endorphins. Additional research is also required to define the precise mechanisms mediating the changes in endogeneous

opioid peptides detected in CSF and the pain relief resulting from stimulation of the periaqueductal gray matter.

YOSHIO HOSOBUCHI
Department of Neurological Surgery, University of California School of Medicine, San Francisco 94143
JEAN ROSSIER
FLOYD E. BLOOM
ROGER GUILLEMIN
Salk Institute, La Jolla, California 92037

References and Notes

1. D. V. Reynolds, *Science* **164**, 444 (1969).
2. J. S. Liebeskind, G. Guilbaud, J. M. Besson, J. L. Oliveras, *Brain Res.* **50**, 441 (1973); J. L. Oliveras, J. M. Besson, G. Guilbaud, J. C. Liebeskind, *Exp. Brain Res.* **20**, 32 (1974); J. L. Oliveras, F. Redjemi, G. Guilbaud, J. M. Besson, *Pain* **1**, 139 (1975); R. Melzack and D. F. Melinkoff, *Exp. Neurol.* **43**, 369 (1974); G. F. Gebhard and J. R. Toleikis, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 439 (1975).
3. S. J. Goodman and V. Holcombe, *Pain* **1**, 264 (1975).
4. D. J. Mayer, T. L. Wolfle, H. Akil, B. Carder, J. C. Liebeskind, *Science* **174**, 1351 (1971); D. J. Mayer and J. C. Liebeskind, *Brain Res.* **68**, 73 (1974).
5. Y. Hosobuchi, J. E. Adams, R. Linchitz, *Science* **197**, 183 (1977).
6. D. E. Richardson and H. Akil, *J. Neurosurg.* **47**, 184 (1977).
7. J. E. Adams, *Pain* **2**, 161 (1976).
8. Y. Hosobuchi and C. H. Li, *Commun. Psychopharmacol.* **2**, 33 (1978).
9. V. Cassinari and C. A. Pagni, *Central Pain* (Harvard Univ. Press, Cambridge, Mass., 1969); R. Garcin, in *Pain*, A. Soulairac, J. Chan, J. Charpentier, Eds. (Academic Press, New York, 1968), p. 521.
10. G. Schaltenbrand and P. Bailey, *Introduction to Stereotaxis with an Atlas of the Human Brain* (Grune and Stratton, New York, 1959), vol. 2.
11. J. E. Adams, Y. Hosobuchi, H. L. Fields, *J. Neurosurg.* **41**, 740 (1974).
12. R. Guillemin, N. Ling, T. Vargo, *Biochem. Biophys. Res. Commun.* **77**, 361 (1977).
13. J. Rossier, T. M. Vargo, S. Minick, N. Ling, F. E. Bloom, R. Guillemin, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5162 (1977).
14. F. Bloom, E. Battenberg, J. Rossier, N. Ling, R. Guillemin, *ibid.* **75**, 1591 (1978).
15. J. D. Bellinzi, N. Grant, V. Garsky, P. Sarantakis, C. D. Wise, L. Stein, *Nature (London)* **260**, 626 (1976); M. Meglio, Y. Hosobuchi, H. H. Loh, J. E. Adams, C. H. Li, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 774 (1977).
16. H. Akil, D. E. Richardson, J. Hughes, J. D. Barchas, *Science* **201**, 463 (1978).
17. All procedures, risks, and benefits pertaining to the ventricular CSF collection during the electrode implantation surgery were explained to all patients participating in this study, and each of them signed a written informed-consent form.
18. This work was supported by grants DA-01785, HD 09690, and AM 18811, as well as by grants from the W. R. Hearst Foundation and the Lorenzo Loh memorial pain-research fund. We thank Raana Azad for expert assistance. J.R. is chargé de recherche, Institut National de la Santé et de la Recherche Medicale, Paris.

30 October 1978

Intracerebral Adrenocorticotropic Hormone Mediates Novelty-Induced Grooming in the Rat

Abstract. *Intact male rats exhibited more grooming in unfamiliar testing chambers than in their home cages. Hypophysectomized rats showed a much reduced increase in grooming in these testing chambers. Intraventricular injections of antiserum to adrenocorticotropic hormone to intact rats decreased the grooming usually observed in the novel situation, whereas a similar injection of control serum did not produce this effect. Peripheral injections of the antiserum did not affect grooming. Since intraventricularly injected adrenocorticotropic hormone induces excessive grooming, these results suggest that the increased grooming observed in the novel environment may be at least partly due to the release of this hormone directly into the cerebral ventricular system.*

Naturally occurring self-grooming in animals has been characterized in a number of different ways by different investigators. Regardless of how it is interpreted, it frequently occurs as an afterreaction to goal-directed actions (*1*), stressful stimulation (*2*), conflict situations (*3*), and fear-induced flight behavior (*4*).

Increased grooming has been observed in the rat in response to local heating of limbic system structures, especially the hypothalamus and septum (*5*), and as an aftereffect of electrical stimulation of the hypothalamus or brainstem (*6*). Low doses of amphetamine (*7*) or morphine (*8*) also induce grooming in rats. Excessive grooming is observed in mice when they are terminating stereotyped responses induced by high doses of amphetamine, that is, 3 to 4 hours after administration (*9*). Whereas peripheral administration of adrenocorticotropic hormone (ACTH) does not induce grooming, intraventricular injection of ACTH elicits excessive grooming (*10–12*). This grooming is not directed toward any particular part of the body, but changes from one portion of the body to another and is complete and effective. It does not appear to be stereotyped, but is an excessively prolonged form of the natural grooming.

We recently observed that when rats were transferred to a novel observation chamber, there was a large increase in the time spent grooming relative to that observed in the home cage, even when similar handling was involved (*12*). To test the hypothesis that the increased grooming observed in the novel testing situation is mediated by ACTH, we examined grooming induced by a novel environment in intact and hypophysectomized rats and in intact rats injected intraventricularly with antiserum to ACTH.

Male Wistar rats (120 to 150 g) were supplied by Charles River (Wilmington, Mass.). Hypophysectomy was performed at the Charles River laboratories on 39-day-old (150 g) rats which were shipped the following day. Rats were housed singly in wire-mesh cages. They were given free access to Purina Lab Chow and water and maintained on a cycle of 12 hours of light and 12 hours of darkness (lights on at 8 a.m.). Ambient temperature under all experimental conditions was 22°C.

The rats were carried 20 m in their home cages to the experimental room and placed in a cage rack similar to the one used in the home room. Transfer to novel observation boxes made of glass and Plexiglas (30 by 30 by 20 cm) occurred in the experimental room. Each box was illuminated by a 7.5-W soft white bulb. The boxes were visually isolated from each other. A white-noise generator provided a continuous masking noise of approximately 65 dB. The rats were always observed in the experimental room with the observer separated from the animals by a one-way mirror. Observation commenced 15 minutes after transport to the experimental room, and continued for a total period of 50 minutes each day at the same time of day (12 noon to 2 p.m.). Whether rats were grooming or not was determined every 15 seconds as described (*11, 12*). On days on which more than one treatment was involved, the observer was unaware of the treatment to particular animals. For statistical analyses we used the Mann-Whitney U test, although in all cases comparable levels of significance were obtained from Student's t-test.

In the first experiment, intact and hypophysectomized rats were scored for grooming in their home cages on days 1 to 3. The rats were then observed in the novel boxes on days 4 to 7, and then again in their home cages on days 8 and 9. Intact animals exhibited a substantial enhancement in their grooming scores on transfer to the novel box (Fig. 1). Grooming in the novel box on day 4 was significantly different from that in the home cage on day 3 ($P < .002$). Average grooming scores for all days in the novel box were higher than those for all days in the home cage ($P < .002$). Over successive days in the novel box this enhanced level of grooming steadily declined, but remained significantly above levels of grooming exhibited in the home cage.

0036-8075/79/0119-0281$00.50