in front of the glass-fronted chamber, unconcealed by any blind (*17*). (iii) Each trial began with the animal's reintroduction into this setting, making "greetings" especially probable. (iv) The vertical rod, intended as a neutral response sensor, provided an almost ideal target for redirected rubbing. Thus, efficiently if inadvertently, the experimenters arranged to evoke the species-typical reactions which they, and many others, failed to recognize and which were construed as evidence for particular learning mechanisms (*18*).

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References and Notes

1. Historically, this tradition reflects, and was reflected in, the transition from Thorndike's era (*2*) to that of Skinner [B. F. Skinner, *Behavior of Organisms* (Appleton, New York, 1938)]. For Thorndike, the instrumental response repertoire was fundamentally a subset of the species-typical repertoire. But Skinner's "operant" responses were said to be of arbitrary form and were defined, not by their topographies, but rather, by their consequences. Thus, whereas Thorndike's system required both observation of subjects and knowledge of their natural behavior, Skinner's required neither.
2. E. L. Thorndike, *Psychol. Rev., Suppl. 2* (1898).
3. It now appears that the operant key-pecking topography arises from Pavlovian conditioning of the grain-pecking reaction and that operant response-shaping techniques are unnecessary [B. R. Moore, in *Constraints on Learning*, R. A. Hinde and J. S. Hinde, Eds. (Academic Press, London, 1973)]. The traditional misinterpretation has also had social implications, in that the impression of technical competence created by the animal research contributed to the spread of operant techniques in psychotherapeutic and related situations.
4. See also R. C. Bolles, *Psychol. Rev.* **77**, 32 (1970).
5. E. R. Guthrie and G. P. Horton, *Cats in a Puzzle Box* (Rinehart, New York, 1946).
6. Thorndike's original monograph (*2*) also dealt with the reactions of cats in puzzle boxes, but his apparatus and techniques were very different from those of Guthrie and Horton, and his data were not compromised.
7. E. R. Guthrie, *The Psychology of Learning* (Peter Smith, Gloucester, Mass., 1960).
8. R. J. Herrnstein, in *Operant Behavior*, W. K. Honig, Ed. (Appleton, New York, 1966).
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10. P. Leyhausen, *Verhaltensstudien an Katzen* (Parey, Berlin, 1973).
11. The term "greeting" is not entirely apt. Although rubbing may indeed be most probable when animals meet, it may also recur from time to time throughout an encounter.
12. The front of the 120 by 85 by 85 cm chamber was constructed of hardware cloth; other surfaces were wooden. No escape door was required. The response sensor was a vertical Lucite tube, 2.5 cm in diameter. Suspended from the ceiling of the chamber, it passed through a small, metallic floor plate, and was clad with copper at that point. The cladding and plate formed a normally open, circular switch which could be closed by a force of 0.05 N. Closure operated remote counters and clocks and drove a ciné camera (Beaulieu R16B) mounted 2 m from the chamber in a heavy sound-attenuating box.
13. Unobtrusive observations were made from a blind by closed-circuit television and by means of switch-operated camera, counters, and clocks.
14. The 2.6- to 3.7-kg adult female cats were obtained from the colony of Dalhousie Medical School, where they had been held briefly for inoculations and quarantine. Between experimental sessions the animals were housed in groups of two or three.
15. A fourth naïve animal, tested briefly, made 6.5 rubbing responses per visit and none between visits. Two other cats were screened, but simply slept in the apparatus. While all four waking cats conformed to the pattern described, it is not universal. Occurrence of the reaction undoubtedly depends upon the nature and timing of previous interactions with humans.
16. Data were presented from only 16 of their 52 cats. A few of these made pawing or biting reactions of possibly Pavlovian origin, or escape reactions resembling those described by Thorndike (*2*). Apart from rubbing, however, the most common reaction was to strike the rod while rolling upon the floor. "Ecstatic" rolling is closely related to rubbing, but seems to occur at higher levels of excitement.
17. According to Guthrie and Horton (*5*) the experimenters and their visitors sat "in full view of the animal. There were at times as many as ten people observing the performance. Conversation was permitted though visitors were warned not to call out to the animals. In ten cases we attempted to coax an inactive animal into activity. Visitors were present during a total of fifteen experiments" (*5*, p. 14).
18. We have not, of course, set out to demonstrate that learning was everywhere irrelevant to the behavior of Guthrie and Horton's cats (*16*). We have argued only that their principal data do not constitute evidence of learning by contiguity (*5*, *7*), "superstition" (*8*), or any other process.
19. Supported by the National Research Council of Canada. Our data were reported at the 1977 Northeastern Regional Meeting of the Animal Behavior Society. We thank R. C. Bolles, H. James, E. P. Lovejoy, and N. J. Mackintosh for comments on an early draft of the manuscript.

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Specific Nonopiate Receptors for β-Endorphin

Abstract. *Iodinated β_H-[2-D-alanine]endorphin exhibits specific binding to cultured human lymphocytes. The binding is inhibited by low concentrations of β-endorphin and its D-alanine² derivative, but is not affected by opiate agonists and antagonists, or by enkephalin analogs, β-lipotropin, adrenocorticotrophic hormone, or α-melanocyte-stimulating hormone; this suggests the existence of a specific, nonopiate binding site (receptor) for β-endorphin. The carboxy-terminal region of β-endorphin is essential for this binding activity, since α-endorphin is not active. β-Endorphin may be a circulating hormone with peripheral physiological effects that are not primarily mediated through interactions with opiate or enkephalin receptors.*

β-Endorphin, which corresponds to the sequence of residues 61 to 91 of β-lipotropin, exhibits a wide range of pharmacological activities in addition to its central analgesic effects (*1*). The typical morphinomimetic effects and some other behavioral effects are blocked by specific opiate antagonists such as naloxone and naltrexone. Among these behavioral effects are excessive grooming behavior (*2*), catatonic states and wet shakes (*3*), and profound sedation and catalepsia (*4*). Some other behavioral effects of β-endorphin, such as the delay in extinction of pole-jumping avoidance (*5*), are not blocked by opiate antagonists and thus would appear to be mediated by actions independent of opiate receptor sites in the brain.

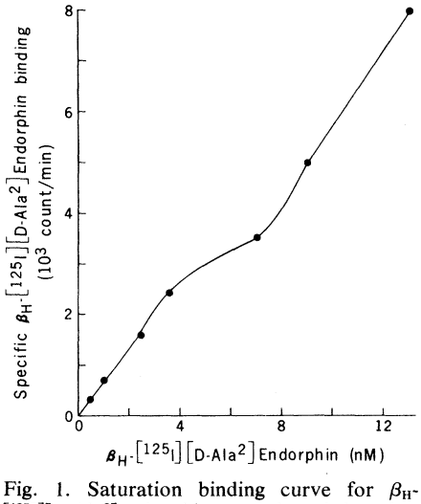

Fig. 1. Saturation binding curve for β_H-[^{125}I][D-Ala^2]endorphin to cultured lymphocytes (10^7 cells per milliliter). The curve exhibits a biphasic function in which binding to low-affinity site is linear up to 150 n*M* (data not shown). Binding was assayed as described for Table 1. Values are the means of three incubations. Very similar data showing such biphasic curves have been obtained in several experiments.

β-Endorphin-like material is present in the serum of rats and its level is increased by acute stress (*6*). Significant levels of β-endorphin are not detectable in normal human serum, although it is present in the plasma of patients with certain endocrine disorders (*7*). In genetically obese mice the pituitary and plasma contents are two to three times greater than in control animals (*8*). The possible nature of the relation between β-endorphin and stress remains highly speculative. Variations in the secretion of β-endorphin by the pituitary or in its circulating plasma levels are not correlated with changes in the cerebral concentration of the peptide (*9*). The stress-induced release of β-endorphin from the pituitary may thus have a physiological role totally distinct from the theoretically possible central analgesic effects of the peptide. Moreover, perfusion of β-endorphin into the pancreas reduces the release of somatostatin and increases the

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secretion of insulin and glucagon (*10*), which further suggests that β-endorphin may be a circulating hormone with special peripheral functions. Furthermore, at least some of these peripheral actions may not be mediated by interactions with opiate receptors.

We have developed a binding assay for iodinated β-endorphin and have examined its interaction with putative opiate receptors in the brain (*11*). This binding is inhibited by opiate agonists and antagonists as well as by very low concentrations of opioid peptides. We now report that β-endorphin can bind specifically to transformed lymphocytes in a manner which is not blocked by enkephalins or opiates; these data suggest the existence of a specific, nonopiate type of binding site (receptor) for β-endorphin.

Transformed cultured human lymphocytes contain specific insulin receptors (*12*), but their physiological significance is not understood. Cultured lymphocytes were selected as a model system for studying β-endorphin receptors because homogeneous populations can be prepared in large quantity and they can be handled without homogenization or purification procedures that may damage the receptor binding activity.

^{125}I-Labeled β_H-[D-Ala^2]endorphin (*13*) shows significant specific binding to cultured lymphocytes (Fig. 1). The binding exhibits a biphasic function, in which the high-affinity site begins to reach a plateau at about 7 n*M*. The apparent K_d value calculated from these data is about 3×10^{-9}*M*. The properties of the low-affinity sites cannot be defined in detail because the high concentrations of labeled ligand used yield high nonspecific binding in this region. However, the inhibition of binding of β_H-[^{125}I][D-Ala^2]endorphin by unlabeled β_H-[D-Ala^2]endorphin and β_H-endorphin (Fig. 2) and the Scatchard plot (Fig. 2, inset) suggest the existence of two different binding sites or negative cooperativity.

The effect of various compounds on the binding of iodinated β_H-[D-Ala^2]endorphin is summarized in Table 1. Opiate agonists and antagonists do not compete with β-endorphin for binding, even at very high concentrations (10^{-5}*M*). Among the enkephalins, only the naturally occurring peptides have some effect (20 to 30 percent inhibition), but at concentrations 1000-fold higher than that of β-endorphin. The metabolically stable enkephalin derivatives have no effect even at these very high concentrations. Moreover, [^{125}I][D-Ala^2, D-Leu^5] enkephalin and [^{125}I][D-Ala^2, (N^{α}-CH$_3$) Phe^4, Met-(*O*)^5-ol] enkephalin (*14*), which bind to opiate receptors very well (*15*), do not bind to these lymphocytes.

The fact that β-lipotropin, adrenocorticotrophic hormone (ACTH), α-melanocyte-stimulating hormone (α-MSH), insulin, and glucagon do not compete for binding further substantiates the implication that the binding site is specific for β-endorphin. Moreover, the COOH-terminal region of β-endorphin, encompassing the sequence H-Leu-Phe-Lys-Asn-Ala-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH (*14*), must be important for the binding interaction since α-endorphin is inactive.

The binding of β_H-[^{125}I][D-Ala^2]endor-

Table 1. Effect of various compounds on the binding of ^{125}I-labeled β_H-[D-Ala^2]endorphin to human cultured lymphocytes (RMPI 6237). The cells (10^7 per milliliter) were incubated with iodinated β_H-[D-Ala^2]endorphin (0.5 n*M*) and different concentrations of the compounds for 45 minutes at 25°C (equilibrium conditions) in a final volume of 0.25 ml of Krebs-Ringer-tris solution, *p*H 7.4. At the end of the incubation the suspension was layered on top of 20 percent sucrose at 4°C in Beckman microfuge tubes and centrifuged for 10 minutes at 40,000*g*. The pellet was severed with scalpel and counted in a γ-emission counter. Specific binding represents the bound radioactivity that can be displaced by 10^{-5}*M* unlabeled β-endorphin.

Compound	Concentration (*M*)	Inhibition of specific binding (%)
β_H-Endorphin	10^{-5}	100
	10^{-7}	72
	10^{-9}	15
Naloxone	10^{-5}	<5
Cyclazocine	10^{-5}	0
Morphine	10^{-5}	0
[Leu^5]Enkephalin	10^{-5}	20
[Met^5]Enkephalin	10^{-5}	30
[D-Ala^2,Leu^5]Enkephalin	10^{-5}	0
[D-Ala^2, (N^{α}-CH$_3$)Phe^4, Met-(*O*)^5-ol]Enkephalin	10^{-5}	0
α-Endorphin	10^{-6}	0
β-Lipotropin	10^{-6}	0
α-MSH	5×10^{-6}	10
ACTH	3×10^{-7}	0
Insulin	10^{-6}	0
Glucagon	10^{-6}	0
Pentalysine	10^{-6}	0

Table 2. Binding of β_H-[^{125}I][D-Ala^2]endorphin to different cell lines. The cells (2 to 10×10^6 per milliliter) were incubated with iodinated β_H-[D-Ala^2]endorphin and specific binding was measured as described for Table 1. Cultured cells were grown in monolayer with 10 percent fetal calf serum and minimum essential medium; RPMI 6237 were grown in suspension with 10 percent fetal calf serum and RPMI 1640. Other cell lines were prepared by usual methods.

Type of binding	Cell line
No specific binding	Fibroblasts (DET 551); ovary cells (CHO); human platelets; rat fat cells; human and rabbit neutrophils; mouse macrophages
Specific binding with low affinity: 100 n*M* < IC_{50} < 1000 n*M*	Fibroblasts [3T3, 3T3(C2), 3T3(SV40)], LM; human lymphocytes
Specific binding with high affinity K_d = 3 n*M*	Cultured human lymphocyte (RPMI 6237)*

*The RPMI 6237 cells contain, in addition, a low-affinity binding site with IC_{50} = 100 n*M*.

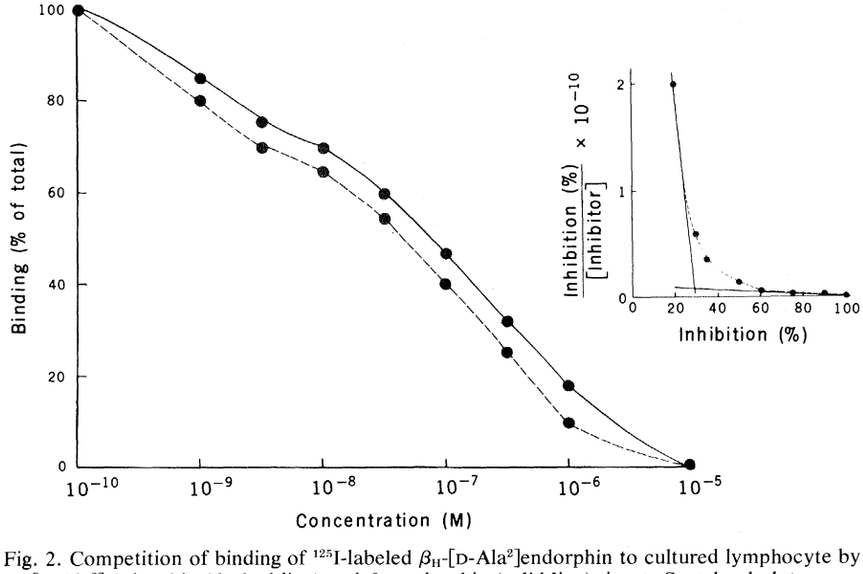

Fig. 2. Competition of binding of ^{125}I-labeled β_H-[D-Ala^2]endorphin to cultured lymphocyte by β_H-[D-Ala^2]endorphin (dashed line) and β_H-endorphin (solid line); inset, Scatchard plot.

phin to different cell lines is summarized in Table 2. Some cell lines do not bind the peptide while other cells only exhibit the low-affinity sites [concentration producing 50 percent inhibition (IC_{50}) between 100 and 1000 nM]. Only transformed lymphocytes appear to have both high-affinity (apparent K_d, 3 nM) and low-affinity (IC_{50} = 100 nM) sites. The high-affinity sites can be destroyed by digestion with trypsin but are resistant to digestion by phospholipase A_2 (data not shown).

Studies of the interaction of iodinated β-endorphin with putative opiate receptors in the brain demonstrate that the binding is inhibited by very low concentrations of enkephalin analogs as well as by opiate agonists and antagonists; this indicates that β-endorphin can bind well to opiate receptors (*11*). However, the nature of the binding interaction is different from that of enkephalins, since various cations affect the binding of β-endorphin and enkephalins markedly differently (*11*). In the present studies β-endorphin binding is not blocked by opiates or enkephalins, which strongly suggests that the β-endorphin binding sites in cultured lymphocytes and in the cell lines exhibiting only the low-affinity sites (Table 2 and data not shown) are quite different from the opiate receptors present in the brain.

β-Endorphin, but not enkephalins, is found mainly in the pituitary gland (*16*) and has a broad range of pharmacological activities. The presence of β-endorphin in serum, the increases induced by stress (*6*), and the existence of specific receptors that do not have the characteristics of opiate receptors suggest that β-endorphin may mediate some central and peripheral physiological functions by mechanisms distinct from those associated with traditional opiate receptors. It will be important to examine other tissues for β-endorphin receptors, and to identify possible pharmacological and physiological effects that are not suppressed by opiate antagonists.

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13. β_H-[D-Ala^2]Endorphin was iodinated by chloramine-T method in the presence of 2M urea and purified on Sephadex G-15 column previously equilibrated and eluted with 0.1M acetic acid (*11*).
14. Amino acid abbreviations: Ala, alanine; Phe, phenylalanine; Met, methionine; Leu, leucine; Lys, lysine; Asn, Asparagine; Ile, isoleucine; Tyr, tyrosine; Gly, glycine; and Glu, glutamic acid.
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Chromosome 13 Long Arm Interstitial Deletion May Result from Maternal Inverted Insertion

Sparkes *et al.* (*1*) reported on a child with an interstitial deletion of chromosome 13 and retinoblastoma. They reported the child's karyotype as 46,XX, del(13)(q14q22) and suggested that the abnormal chromosome was derived from the mother's normal chromosome 13. The maternal homolog was reported to carry a simple paracentric inversion with break points in bands q12 and q22. Sparkes *et al.* hypothesized that the derived chromosome present in the daughter may have resulted from the formation of two breaks followed by rehealing in a prophase I inversion loop.

I suggest that the mother may instead have an inverted insertion with a karyotype of 46,XX,inv ins(13)(pter→q12; :q22→q14::q12→q14::q22→qter). The inverted insertion chromosome would appear indistinguishable from the inversion suggested by Sparkes *et al.* when stained by the G and Q banding techniques that these workers used (Fig. 1).

Such a chromosome, when paired with its normal homolog, would form a double loop during prophase I similar to the loops resulting from overlapping inversions in *Drosophila* (*2*). A single crossover within the noninverted interstitial segment would then produce a chromosome with an interstitial deletion of the type found in the child (Fig. 2A).

It is generally recognized that in humans inverted insertions will also produce duplication and deletion chromosomes by crossing over. Palmer *et al.* (*3*) studied a proband with a duplicated segment of chromosome 1. Her father carried an inverted insertion of that chromo-

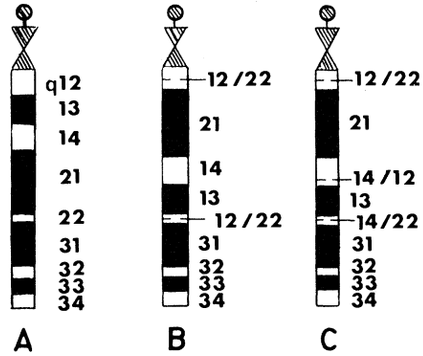
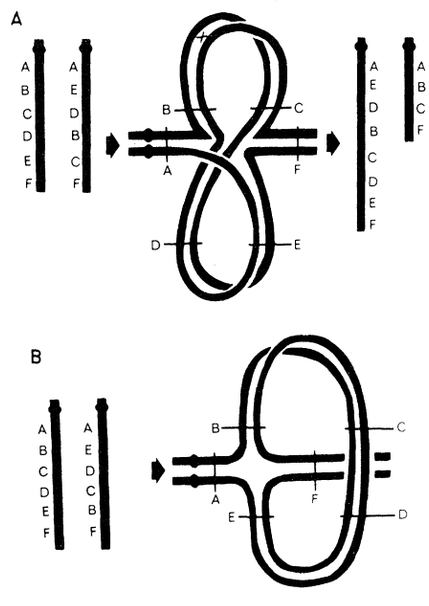


Fig. 1 (left). Chromosome 13. (A) Normal 13. (B) Inversion of bands q12 to q22. (C) Inverted insertion with break points at q12, q14, and q22. Fig. 2 (right). Pachytene inversion loops. Two of four strands are shown. (A) An inverted insertion. A single crossover within the inserted portion will produce deleted and duplication products. (B) Inversion loop from a paracentric inversion drawn in three-dimensional perspective.



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