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Antagonism of Stress-Induced Analgesia by D-Phenylalanine, an Anti-Enkephalinase

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BODNAR, R. J., M. LATTNER AND M. M. WALLACE. *Antagonism of stress-induced analgesia by D-phenylalanine, an anti-enkephalinase.* PHARMAC. BIOCHEM. BEHAV. 13(6) 829–833, 1980.—Methionine- and leucine-enkephalin produce mild and transient analgesic effects, presumably because of enzymatic degradation. Administration of high (250 mg/kg) doses of D-phenylalanine retards the degradation process and elicits analgesia which is reversed by naloxone and which summates with electroacupuncture analgesia. The present study evaluated D-phenylalanine's dose-dependent effects upon a non-opioid analgesic treatment, cold-water swims (CWS), and compared this with morphine. Following determination of flinch-jump baselines, three groups of rats received respectively either 25, 50 or 100 mg/kg of D-phenylalanine intraperitoneally in three conditions: alone, with CWS (2°C for 3.5 min), and with morphine (5 mg/kg, SC). Parallel controls with saline were also tested. Simultaneous exposure with each minimally analgesic dose of D-phenylalanine reduced significantly the analgesic, but not hypothermic effects of CWS. By contrast, morphine analgesia was unaffected by D-phenylalanine. These data provide further support that different pain-inhibitory systems mediate CWS and morphine analgesia and suggest that activation of one system is capable of exerting collateral inhibition upon the other.

Pain Analgesia Enkephalins D-Phenylalanine Cold-water swims Morphine Collateral inhibition

THE two endogenous pentapeptides with opiate properties, methionine (met)-enkephalin and leucine (leu)-enkephalin [31,32], appeared to be ideal agents for pain relief. Yet, intraventricular injections of high doses of each pentapeptide induced only mild and transient analgesic effects [2,28], presumably because they were degraded rapidly by such enzymes as carboxypeptidase A and leucine amino peptidase [15]. Development of more stable enkephalin analogues, such as (D-Ala)^2-met-enkephalin [33,39], induced more potent and longer-lasting analgesic effects [38,43]. Another method of prolonging the analgesic efficacy of these peptides is to selectively inhibit enkephalinase enzymes and thereby prevent degradation of enkephalins in the central nervous system. Administration of D-phenylalanine, which posesses anti-nociceptive properties in rodents and man [21], inhibits carboxypeptidase A and leucine amino peptidase in both mouse and guinea pig brain [22]. The analgesic effects of D-phenylalanine do not develop tolerance, but are reversed by high doses of the opiate antagonist, naloxone [23]. Moreover, D-phenylalanine analgesia and the endogenous content of endorphins in different mouse strains correlate positively [16]. The endorphin-rich Ob/Ob strain [35] exhibits greater analgesia following D-phenylalanine than normal B6AF$_{1/J}$ mice which in turn display greater analgesia than the endorphin-poor CXBK strain [1].

Cheng and Pomeranz [18] also demonstrated that the analgesic effects of D-phenylalanine and electroacupuncture summate, and that this effect is reversed by naloxone. These data support their contention that electroacupuncture analgesia (EAA) in mice is mediated by endogenous opioid processes. EAA, particularly in low frequencies [17], is reversible by levo-naloxone and other opiate antagonists but not by dextro-naloxone [19]. Also, pituitary endorphins appear to mediate EAA since hypophysectomy, dexamethasone injections and 2% saline treatment reduce this analgesic effect [20,40].

Acute exposure to cold-water swims (CWS) in rats also elicits analgesia [5] which, like EAA, is attenuated by hypophysectomy [4]. The anterior lobe of the pituitary appears to be responsible for this effect since removal of the posterior lobe does not diminish CWS analgesia [26] while adrenalectomy potentiates this analgesic effect [27]. Yet, CWS analgesia and EAA display some important differences. First, CWS analgesia is not reversed by low doses of naloxone with only partial effects at high doses [7]. Second, p-chlorophenylalanine, which blocks high-frequency EAA [17], does not affect CWS analgesia [11]. Third, 2% saline treatment does not affect CWS analgesia [10]. Therefore, to assess further whether endogenous opioid mechanisms are involved in mediating CWS analgesia, the present study examined in rats the effects of D-phenylalanine administration upon CWS analgesia and also morphine analgesia.

EXPERIMENT 1

Method

Twenty-four male, albino Sprague-Dawley rats (200–300 g) were tested for flinch-jump thresholds using a modification of the Evans procedure [24]. Electric shocks were delivered through a 30-cm by 24-cm floor composed of 16 grids by a 60-Hz constant current shock generator and an electromechanical grid scrambler. Using an ascending method of limits of successively more intense shocks, the flinch threshold was defined in mA as the lowest intensity that

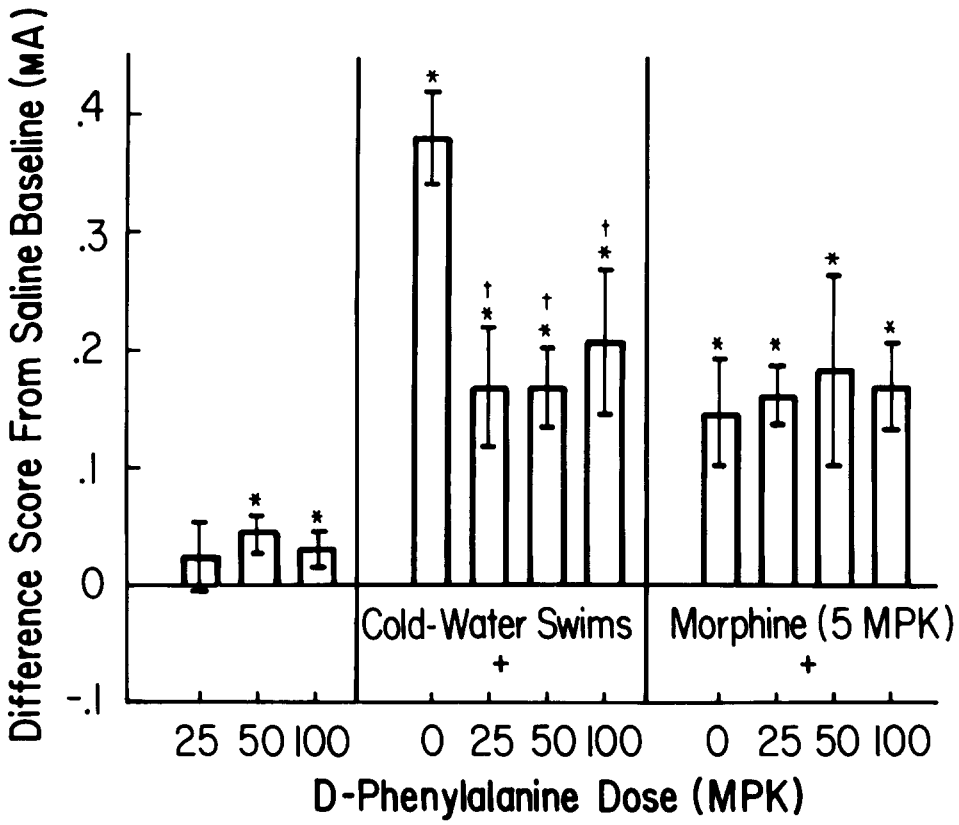

FIG. 1. Alterations in mean jump thresholds (±SEM) following either administration of different doses (0, 25, 50, 100 mg/kg, IP) of D-phenylalanine alone, or in conjunction with either 2°C cold-water swims (CWS) for 3.5 min or morphine (5 mg/kg, SC). The asterisks denote a significant difference from saline baseline at the 0.05 level, while the crosses denote a significant difference from the cold-water swim (0) condition.

elicited a withdrawal of a single paw from the grids. The jump threshold was defined as the lowest of two consecutive intensities that elicited simultaneous withdrawal of both hindpaws from the grids. Each trial began with the animal receiving a 300-msec foot shock at a current intensity of 0.1 mA. Subsequent shocks occurred at 10-sec intervals and were increased in equal 0.05 mA steps until each nociceptive threshold was determined. After each trial, the current intensity was reset to 0.1 mA for the next trial until 6 trials were completed. Daily flinch and jump thresholds were each computed as the mean of these six trials. The experimenter conducting the flinch-jump test was uninformed of the purpose of the experiment.

Based on the mean of four days of stable baseline thresholds, rats were assigned into three matched groups of eight rats each. Each group was exposed to the following six experimental conditions: (a) an injection of D-phenylalanine (Sigma: 25 mg/ml normal saline, IP) 30 min before the flinch-jump test; (b) an injection of an equimolar amount of saline 30 min before the flinch-jump test; (c) an injection of D-phenylalanine 35 min prior to *and* a single 3.5-min CWS at 2°C 30 min prior to the flinch-jump test; (d) an injection of saline 35 min prior to *and* a CWS 30 min prior to the flinch-jump test; (e) an injection of D-phenylalanine 35 min prior to *and* a single injection of morphine (5 mg morphine sulfate/ml buffered solution/kg body weight, SC) 30 min prior to the flinch-jump test; and (f) an injection of saline 35 min prior to *and* an injection of morphine 30 min prior to the flinch-jump test. The order of experimental conditions within each group was determined by a Latin Square design to control for order and carry-over effects with a minimum of 48 hr elapsing between each condition. The dose of D-phenylalanine varied across groups: group 1 received 25 mg/kg; group 2 received 50 mg/kg; and group 3 received 100 mg/kg. Corresponding saline injections controlled for changes in volume across doses.

Results

Figure 1 shows that D-phenylalanine, CWS and morphine each increased jump thresholds relative to saline controls. However, when CWS was paired with each dose of D-phenylalanine, the analgesic efficacy of CWS was diminished. By contrast, when morphine was paired with each dose of D-phenylalanine, the analgesic effects of morphine were unaffected. A two-way split-plot analysis of variance revealed significant differences across the baseline and experimental conditions, $F(6,126)=216.50$, $p<0.01$, and for the dose by condition interaction, $F(12,126)=2.50$, $p<0.01$, but not across the three doses, $F(2,21)=0.36$. Pairwise Tukey post-hoc comparisons were made since each subject served as its own control. First, jump thresholds following saline did not differ significantly from baseline values for any group. Therefore, all further comparisons were made between a

TABLE 1

ALTERATIONS IN CORE TEMPERATURE (°C) FOLLOWING COLD-WATER SWIMS IN D-PHENYLALANINE AND SALINE TREATED RATS

Group	n		BL	Post-swim (min) 0	15	30	60	120
D-phenylalanine (100 mg/kg)	6	mean	38.1	30.9	25.9	27.8	31.7	36.4
		SEM	0.1	0.6	0.4	0.6	0.9	0.9
		F vs BL	—	99.7	604.9	237.2	35.3	2.6
		p	—	<0.01	<0.01	<0.01	<0.01	NS
Saline	6	mean	38.1	31.7	26.3	28.3	32.4	37.2
		SEM	0.1	0.6	0.4	0.5	0.5	0.3
		F vs BL	—	77.7	620.6	232.1	74.8	6.6
		p	—	<0.01	<0.01	<0.01	<0.01	<0.05
D-phenylalanine vs saline		F	0.0	0.6	0.3	0.4	0.3	0.6
		p	NS	NS	NS	NS	NS	NS

given experimental condition and the saline control. Second, D-phenylalanine produced small, but significant increases in jump thresholds as compared to saline at the 100 mg/kg, $t(7)=1.93$, $0.01>p>0.05$, and 50 mg/kg ($t=2.44$, $p<0.05$) doses, but not following the 25 mg/kg dose ($t=0.84$). Third, jump thresholds were increased significantly following CWS paired with saline, $t(23)=8.84$, $p<0.01$, and with D-phenylalanine at doses of 25 mg/kg, $t(7)=4.71$, $p<0.01$, 50 mg/kg ($t=3.88$, $p<0.01$) and 100 mg/kg ($t=7.17$, $p<0.01$). However, the analgesic effects of CWS were significantly diminished when the swims were paired with D-phenylalanine at doses of 25 mg/kg ($t=2.89$, $p<0.05$), 50 mg/kg ($t=1.99$, $0.10>p>0.05$) and 100 mg/kg ($t=2.51$, $p<0.05$) than when the swims were paired with saline. Fourth, jump thresholds were increased significantly following morphine paired with saline, $t(23)=5.21$, $p<0.01$, and with D-phenylalanine at doses of 25 mg/kg, $t(7)=5.77$, $p<0.01$), 50 mg/kg ($t=2.07$, $0.10>p>0.05$) and 100 mg/kg ($t=4.03$, $p<0.01$). No significant changes in morphine analgesia occurred following D-phenylalanine pairing.

EXPERIMENT 2

The finding that CWS analgesia is reduced significantly following D-phenylalanine administration can evoke many other interpretations other than D-phenylalanine's effects upon the analgesic system(s) activated by CWS. One strong possibility is that D-phenylalanine may alter the rat's perception of the stressful consequences of CWS. In previous studies where the analgesic effects of CWS have been diminished by either adaptation [8] or hypophysectomy [4], we found that such reductions were not due to alterations in the animal's perceptions of hypothermia. Thus, if D-phenylalanine is exerting its effects upon CWS by altering its efficacy as a stressor, then parallel alterations would be expected in core body temperature following CWS in D-phenylalanine treated animals.

Method

One group of six rats received an intraperitoneal injection of 100 mg/kg of D-phenylalanine 5 min prior to CWS while a second matched group received a saline injection 5 min before CWS. Core body temperatures for each animal were determined before the injection and swim, and then 0, 15, 30, 60 and 120 min following CWS. Core body temperatures were measured with the rectal probe of a Bailey digital thermometer (BAT-8).

Results

A two-way analysis of variance revealed significant differences across the pre- and post-CWS time course, $F(5,60)=146.54$, $p<0.01$, but not between groups, $F(1,60)=2.81$, or for the group by time interaction, $F(5,60)=0.15$. Table 1 summarizes the hypothermic time course of CWS for each group, and shows that at each post CWS temperature determination, the D-phenylalanine and saline groups did not differ in their hypothermic responses.

DISCUSSION

The present study demonstrated that pretreatment with the mild analgesic, D-phenylalanine, significantly reduced the analgesic response to CWS without affecting its hypothermic response. By contrast, morphine analgesia is unaffected by this particular dose range of D-phenylalanine. These data support previously-reported dissociations between CWS and morphine analgesia, including: (a) lack of cross-tolerance [9]; (b) elimination of morphine, but not CWS, analgesia by naloxone [7,36]; (c) hypophysectomy's attenuation of CWS analgesia [4] and potentiation of morphine analgesia [6,30]; (d) elimination of CWS, but only attenuation of morphine analgesia in rats treated neonatally with monosodium glutamate [3]; (e) attenuation of morphine [11,41], but not CWS analgesia [11] following p-chlorophenylalanine administration; and (f) elimination of CWS, but not morphine analgesia in Brattleboro rats with diabetes insipidus [12].

Though CWS analgesia and EAA are dependent upon intact pituitary processes [4,40], the latter, but not the former treatment is apparently subserved by pituitary endorphins [17, 18, 19, 20, 40]. D-phenylalanine, by inhibiting carboxypeptidase A and leucine amino peptidase [22], appear to prolong the lifetime of enkephalins and endorphins released by EAA, thereby enhancing its analgesic effects. Opiate re-

ceptor involvement is supported by naloxone reversal of D-phenylalanine's and electroacupuncture's summative effect [18]. The present data indicated that morphine and D-phenylalanine doses of 25, 50 and 100 mg/kg did not summate. However, this dose range, used primarily to indicate the CWS effects, is considerably lower than the 250 mg/kg dose employed to show D-phenylalanine's anti-nociceptive [21,22] and summative properties with EAA [18]. Alternatively, morphine analgesia and EAA may be subserved by different endogenous opioid sites since hypophysectomy potentiates the former effect [7,30] while attenuating the latter [40].

The remaining question as to why the anti-enkephalinase, D-phenylalanine antagonizes CWS analgesia might be explained in terms of the recent proposals [9, 14, 29, 37] that at least two pain-inhibitory systems exist. One system, responsible in large part for the analgesic effects of morphine and electrical brain stimulation, consists of an interaction between the endogenous opioid systems and the descending bulbospinal serotonergic system [25]. The second system, apparently responsible for the analgesic effects of certain stressors, seems to be relatively independent of endogenous opioid functions [3, 4, 6, 7, 9, 11, 12] and consists in part of the anterior lobe of the pituitary gland and medial-basal hypothalamus. A possible third system in the spinal cord to mediate opiate analgesia has also been described [44]. The process by which these systems are activated may explain the present and other paradoxical results. Activation of all means of alleviating pain in response to a particular nociceptive stimulus would appear to be a maladaptive act since pain relief is an essential feature of an organism's survival. Use of all pain-inhibitory systems would leave the animal with no reserve capability. Therefore, it would be both adaptive and parsimonious if activation of one pain-inhibitory system resulted in the inhibition of other pain-inhibitory systems. The decision as to which pain-inhibitory system may be dependent upon the endogenous and exogenous environmental context of the nociceptive stimulus. As described in the classic studies of Cannon [13], nociceptive stimuli can evoke a large range of overall behavioral responses from defensive aggression to withdrawal and recuperation. The present data provide some support for concurrent inhibition in that the larger resultant available pool of enkephalins and endorphins following D-phenylalanine treatment may act to inhibit the stress-related pain-inhibitory system and thereby reduce the analgesic effectiveness of CWS. Conversely, removal of an integral component of one pain-inhibitory system should act to disinhibit the other systems. For instance, removal of the pituitary gland, an integral component of the stress-related pain-inhibitory system, potentiates morphine analgesia [6,30]. Similarly, p-chlorophenylalanine, which reduces brain serotonin [34] and removes an integral component of the opioid system, prolongs the analgesic effects of inescapable foot shock [11,42]. Whether concurrent inhibition indeed exists among pain-inhibitory processes is still highly speculative, but such a notion may hold great promise for possible treatments for pain relief and provide a workable model for analgesic processes.

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REFERENCES

1. Baran, A., L. Shuster, B. E. Eleftheriou and D. W. Bailey. Opiate receptors in mice: genetic differences. *Life Sci.* **17:** 633–640, 1975.
2. Belluzzi, J. D., N. Grant, V. Garsky, V. D. Sarantakis, C. D. Wise and L. Stein. Analgesia induced in vivo by central administration of enkephalin in rat. *Nature* **260:** 625–626, 1976.
3. Bodnar, R. J., G. W. Abrams, E. A. Zimmerman, D. T. Krieger, G. Nicholson and J. S. Kizer. Neonatal monosodium glutamate: effects upon analgesic responsivity and immunocytochemical ACTH/B-lipotropin. *Neuroendocrinology* **30:** 280–284, 1980.
4. Bodnar, R. J., M. Glusman, M. Brutus, A. Spiaggia and D. D. Kelly. Analgesia induced by cold-water stress: attenuation following hypophysectomy. *Physiol. Behav.* **23:** 53–62, 1979.
5. Bodnar, R. J., D. D. Kelly and M. Glusman. Stress-induced analgesia: time course of pain reflex alterations following cold-water swims. *Bull. Psychon. Soc.* **11:** 333–336, 1978.
6. Bodnar, R. J., D. D. Kelly, A. Mansour and M. Glusman. Differential effects of hypophysectomy upon analgesia induced by two glucoprivic stressors and morphine. *Pharmac. Biochem. Behav.* **11:** 303–308, 1979.
7. Bodnar, R. J., D. D. Kelly, A. Spiaggia, C. Ehrenberg and M. Glusman. Dose-dependent reductions by naloxone of analgesia induced by cold-water stress. *Pharmac. Biochem. Behav.* **8:** 667–672, 1978.
8. Bodnar, R. J., D. D. Kelly, A. Spiaggia and M. Glusman. Stress-induced analgesia: adaptation following chronic cold water swims. *Bull. Psychon. Soc.* **11:** 337–340, 1978.
9. Bodnar, R. J., D. D. Kelly, S. S. Steiner and M. Glusman. Stress-produced analgesia and morphine-produced analgesia: lack of cross-tolerance. *Pharmac. Biochem. Behav.* **8:** 661–666, 1978.
10. Bodnar, R. J., D. D. Kelly, L. W. Thomas and M. Glusman. Two % saline treatment: failure to alter opiate and cold-water stress analgesia. *Physiol. Behav.* **24:** 805–806, 1980.
11. Bodnar, R. J., M. M. Wallace, J. H. Kordower, A. Kirchgessner, D. Simone. K. Merrigan, J. Scalisi and M. Lattner. Analgesic responses to stress and opiates following selective destruction of either catecholaminergic, serotonergic or Substance P pathways. *Soc. Neurosci. Abstr.* **6:** in press, 1980.
12. Bodnar, R. J., E. A. Zimmerman, G. Nilaver, A. Mansour, L. W. Thomas, D. D. Kelly and M. Glusman. Dissociation of cold-water swim and morphine analgesia in Brattleboro rats with diabetes insipidus. *Life Sci.* **26:** 1581–1590, 1980.
13. Cannon, W. B. *The Wisdom of the Body*. New York: Norton, 1939.
14. Chance, W. T., G. M. Krynock and J. A. Rosecrans. Antinociception following lesion-induced hyperemotionality and conditioned fear. *Pain* **4:** 243–252, 1978.
15. Chang, J. K., B. T. W. Fong, A. Pert and C. B. Pert. Opiate receptor affinities and behavioral effects of enkephalin: structure-activity relationship of ten synthetic peptide analogues. *Life Sci.* **18:** 1473–1482, 1976.
16. Cheng, R. S. S. and B. Pomeranz. Correlation of genetic differences in endorphin systems with analgesic effects of D-amino acids in mice. *Brain Res.* **177:** 583–587, 1979.
17. Cheng, R. S. S. and B. Pomeranz. Electroacupuncture analgesia could be mediated by at least two pain-relieving mechanisms: endorphin and non-endorphin systems. *Life Sci.* **25:** 1957–1962, 1979.
18. Cheng, R. S. S. and B. Pomeranz. A combined treatment with D-amino acids and electroacupuncture produces a greater analgesia than either treatment alone; naloxone reverses these effects. *Pain* **8:** 231–236, 1980.

19. Cheng, R. S. S. and B. Pomeranz. Electroacupuncture analgesia is mediated by stereospecific opiate receptors and is reversed by antagonists of type I receptors. *Life Sci.* **26:** 631–638, 1980.
20. Cheng, R., B. Pomeranz and G. Yu. Dexamethasone partially reduces and 2% saline-treatment abolished electro-acupuncture analgesia: these findings implicate pituitary endoephins. *Life Sci.* **24:** 1481–1486, 1979.
21. Ehrenpreis, S., R. C. Balagot, J. E. Comaty and S. Myles. Naloxone reversible analgesia in mice produced by D-phenylalanine and hydrocinnamic acid inhibitors of carboxypeptidase A. In: *Advances in Pain Research and Therapy*, Vol. 3, edited by J. J. Bonica, J. C. Liebeskind and D. G. Albe-Fessard. New York: Raven, 1979.
22. Ehrenpreis, S., R. C. Balagot, S. Myles, C. Advocate and J. E. Comaty. Further studies on the analgesic activity of D-phenylalanine (DPA) in mice and humans. In: *Endogenous and Exogenous Opiate Agonists and Antagonists*, edited by E. L. Way. New York: Pergamon, 1980.
23. Ehrenpreis, S., J. E. Comaty and S. B. Myles. Naloxone reversible analgesia produced by D-phenylalanine in mice. *Soc. Neurosci. Abstr.* **4:** 459, 1978.
24. Evans, W. O. A new technique for the investigation of some analgesic drugs on a reflexive behavior in the rat. *Psychopharmacologia* **2:** 318–325, 1961.
25. Fields, H. L. and A. I. Basbaum. Brain control of spinal pain transmission neurons. *A. Rev. Physiol.* **40:** 217–248, 1978.
26. Glusman, M., R. J. Bodnar, D. D. Kelly, C. Sirio, J. Stern and E. A. Zimmerman. Attenuation of stress-induced analgesia by anterior hypophysectomy in the rat. *Soc. Neurosci. Abstr.* **5:** 609, 1979.
27. Glusman, M., R. J. Bodnar, A. Mansour and D. D. Kelly. Enhancement of stress-induced analgesia by adrenalectomy in the rat. *Soc. Neurosci. Abstr.* **6:** in press, 1980.
28. Graf, L., J. I. Szekely, A. Z. Ronai, Z. Dunai-Kovacs and S. Bajusz. Comparative study on analgesic effect on Met^5-enkephalin and related lipotropin fragments. *Nature* **263:** 240–241, 1976.
29. Hayes, R. L., G. J. Bennett, P. G. Newlon and D. J. Mayer. Behavioral and physiological studies of non-narcotic analgesia in the rat elicited by certain environmental stimuli. *Brain Res.* **155:** 69–90, 1978.
30. Holaday, J. W., P. Y. Law, L. F. Tseng, H. H. Loh and C. H. Li. B-endorphin: pituitary and adrenal glands modulate its action. *Proc. natn. Acad. Sci. U.S.A.* **74:** 4628–4632, 1978.
31. Hughes, J. Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. *Brain Res.* **88:** 295–308, 1975.
32. Hughes, J., T. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan and H. R. Morris. Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* **258:** 577–579, 1975.
33. Kastin, A. J., C. Nissen, A. V. Schally and D. H. Coy. Blood-brain barrier, half-time disappearance and brain distribution for labeled enkephalin and a potent analog. *Brain Res. Bull.* **1:** 583–589, 1976.
34. Koe, B. K. and A. Weissman. P-chlorophenylalanine: a specific depletor of brain serotonin. *J. Pharmac. exp. Ther.* **154:** 499–516, 1966.
35. Margules, D. L., M. Beatriz, M. J. Lewis, H. Shibuya and C. B. Pert. B-endorphin is associated with overeating in genetically obese mice (Ob/Ob) and rats (fa/fa). *Science* **202:** 988–991, 1978.
36. Martin, W. R. Opioid antagonists. *Pharmac. Rev.* **19:** 463–521, 1967.
37. Mayer, D. J. and D. D. Price. Central nervous system mechanisms of analgesia. *Pain* **2:** 379–404, 1976.
38. Pert, A. Behavioral pharmacology of [$D\text{-}Ala^2$]-methionine-enkephalin amide and other long-acting opiate peptides. In: *Opiates and Endogenous Opioid Peptieds*, edited by H. W. Kosterlitz. Amsterdam: North-Holland, 1976.
39. Pert, C. B., A. Pert, J. K. Change and B. T. W. Fong. [$D\text{-}Ala^2$]-met-enkephalinamide: a potent long lasting synthetic peptide analgesic. *Science* **194:** 330–332, 1976.
40. Pomeranz, B., R. Cheng and P. Law. Acupuncture reduces electrophysiological and behavioral responses to noxious stimuli: Pituitary is implicated. *Expl Neurol.* **54:** 172–178, 1977.
41. Tenen, S. S. Antagonism of the analgesic effect of morphine and other drugs by p-chlorophenylalanine, a serotonin depletor. *Psychopharmacologia* **12:** 278–285, 1968.
42. Tricklebank, M. D., P. H. Hutson and G. Curzon. Footshock-induced hyporesponsiveness to noxious stimuli: Decreased by 5HT but not increased by 5HT antagonists. Submitted for publication.
43. Walker, J. M., G. G. Berntson, C. A. Sandman, D. H. Coy, A. V. Schally and A. J. Kastin. An analog of enkephalin having prolonged opiate-like effects *in vivo*. *Science* **196:** 85–87, 1977.
44. Yaksh, T. L. and T. A. Rudy. Narcotic analgetics: CNS sites and mechanisms of action as revealed by intracerebral injection techniques. *Pain* **4:** 299–359, 1978.