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Opiate Dependence and Withdrawal—A New Synthesis?

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HENDRIE, C. A. *Opiate dependence and withdrawal—A new synthesis?* PHARMACOL BIOCHEM BEHAV **23**(5) 863–870, 1985.—There is a growing body of evidence to suggest that adrenocorticotropin (ACTH) may have a physiological role as an endogenous contra-opioid agonist. In addition to having appreciable affinity for opiate receptors and inducing many behavioural and intracellular effects opposite to those observed following opioid administration, ACTH may interact with endorphins in a mutually antagonistic manner. On the basis of these data a model of opiate dependence is proposed whereby several aspects of the opiate abstinence syndrome may be attributed to the excitatory actions of ACTH acting at opiate receptors. Thus, it may be predicted that opiate antagonist administration during primary abstinence should significantly attenuate many aspects of this behavioural syndrome. The present study was conducted in order to investigate this hypothesis. Results inicated that whilst naloxone (1.5 mg/kg) exerted little influence in non-dependent animals, it significantly attenuated abstinence-exacerbated grooming, body shaking, teeth chattering and sneezing, in addition to completely antagonizing withdrawal hyperalgesia in post-dependent animals. These data are consistent with the proposed existence of an endogenous contra-opioid ligand, the antagonism of which markedly reduces the severity of the morphine withdrawal syndrome.

Morphine abstinence Naloxone Opiates Opioids Contra-opioids

IN view of the high abuse potential of opiates such as morphine [23], coupled with the difficulty in weaning addicts off such drugs, the isolation and identification of the endogenous opioid peptides [14,33] heralded great hopes for the development of a new class of powerful non-addictive analgesics. However, even before the end of the 1970's, it had become apparent that this was not to be the case, with several groups reporting the develpment of tolerance and dependence following chronic administration of both β-endorphin [69, 73, 74] and synthetic enkephalin analogues [8, 15, 80, 81], in addition to their cross-tolerance with morphine [45, 55, 77]. Further, tolerance has been shown to develop following even a single injection of β-endorphin [35] whilst naloxone is able to precipitate withdrawal after only 24 hours of repeated peptide administration [32, 60, 86, 87]. Thus, as it has become increasingly unlikely that synthetic opioids will be of clinical usefulness as non-addictive analgesics, the need to develop a greater understanding of the mechanisms of tolerance and dependence *per se* remains undiminished.

The finding that naloxone can precipitate withdrawal in dependent animals [82], and inhibit the development of tolerance and dependence when administered concomitantly with opiate agonists [10,34], strongly indicates the involvement of opiate receptors in these phenomena. Therefore several authors have postulated that exogenous opiates induce tolerance and dependence through an interaction with endogenous opioidergic mechanisms [23, 31, 39]. For example, Kosterlitz and Hughes [39] have suggested that exogenous opiates inhibit the synthesis and release of endorphins. The resultant decrease in opioid production induces a need for greater dosages of opiate in order to maintain homeostasis. Levels of endorphins are consequently further reduced, and thus a vicious cycle is developed. Upon cessation of drug regimen, the dependent system is temporarily devoid of all opiate or opioid inhibitory influences and thereby enters into a state of withdrawal. Similarly, Gold *et al.* [22] propose that the functional integrity of endorphinergic mechanisms in the locus coeruleus may be compromised by chronic opiate administration. In this instance, withdrawal is seen as being the result of the release of neurotransmitters more usually under endorphinergic inhibitory control.

Thus, on the view that withdrawal is the consequence of the *absence* of endorphins, it has been suggested that only opioid agonists may supress the abstinence syndrome [44]. However, in contrast to the above view, several lines of evidence now indicate that the opiate withdrawal syndrome may instead be mediated by the *presence* of an endogenous ligand which competes with and has functional consequences opposite to those normally ascribed to opiates. Evidence further suggests that this "contra-opioid agonist" [27] may be ACTH.

Firstly, ACTH induces many behavioural alterations that are in direct contrast to those induced by β-endorphin. For example, ACTH stimulates sexual behaviour [5,7] and induces hyperalgesia [6] whilst β-endorphin inhibits sexual responding [46,47] and is a powerful analgesic [9, 68, 70]. Similarly, at the intracellular level, $ACTH_{1-24}$ stimulates adenylate cyclase activity leading to increased levels of cyclic 3′, 5′-adenosine monophosphate (cAMP) [57, 83, 84] whilst opiates inhibit adenylate cyclase and prostaglandin-E stimulated cAMP formation [13, 26, 49, 59, 88]. Secondly, in addition to the well documented relationship between ACTH and β-endorphin, both in terms of synthesis and aspects of their

distribution (for review see [51]), many effects of ACTH have been found to be naloxone-reversible [21,38]. These data suggest an action of ACTH at opiate receptors, a view supported by demonstrations that $ACTH_{1-28}$, $ACTH_{1-24}$ and $ACTH_{4-10}$ have appreciable affinity for opiate binding sites [1, 64, 66]. Thirdly, β-endorphin has been found to antagonise $ACTH_{1-24}$ induced yawning/stretching syndrome, spontaneous penile erections and hyperalgesia [6,18]. Conversely, $ACTH_{1-24}$ attenuates β-endorphin-induced analgesia [2,3], catalepsy [18] and elevated prolactin levels [16] in addition to curtailing opioid-mediated environmentally-induced alterations in nociception [24]. In view of these findings, it has been suggested that ACTH may have a physiological role as an endogenous opioid antagonist [2, 6, 65]. In accordance with this hypothesis ACTH has been shown to competitively inhibit opiate binding in vitro [40, 66, 85] whilst $ACTH_{1-24}$ selectively displaces β-endorphin from opiate receptors in a dose dependent manner [1]. Finally, whilst observations of parallel increases in stress-induced ACTH and β-endorphin secretion have led to the conclusion that these peptides are released concomitantly [17, 25, 42, 75], there is now evidence to suggest that they be under mutual physiological control, each influencing the release of the other. Opiates or opioids stimulate ACTH activity [37, 43, 48, 72, 75] whilst ACTH or corticosteroids inhibit β-endorphin output [56,61]. Thus, it is postulated that a feedback mechanism exists between these peptides. β-endorphin inducing ACTH release, which in turn (perhaps via corticosteroids) inhibits β-endorphin. On this view, whilst there would be a short time-lag between stimulated β-endorphin and ACTH output (i.e. [52]), these peptides could appear to be increasing and decreasing in parallel.

In summary, ACTH has affinity for opiate receptors, interacts with β-endorphin in a mutually antagonistic manner and induces many behavioural and cellular effects opposite to those observed following opioid administration. Together, these data suggest a physiological role for ACTH as an endogenous contra-opioid agonist (i.e., a ligand interacting with opiate receptors but producing detectable effects opposite to those induced by the traditional receptor agonist). Further, these findings may also indicate a significant influence of ACTH in the mediation of opiate tolerance and dependence. On the basis of these data, a model of dependence is proposed whereby many aspects of the opiate abstinence syndrome may be attributed to the excitatory actions of ACTH. A schematic representation of this model is presented in Fig. 1.

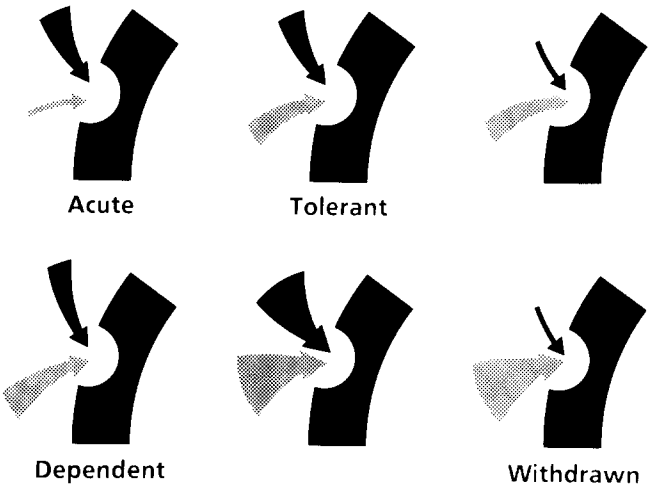

FIG. 1. Model of opiate tolerance, dependence and withdrawal. Schematic representation of proposed mechanism for opiate tolerance, dependence and withdrawal. (White semi circle=opiate receptor; black arrows=opioid; hatched arrows=ACTH). In situations where the ACTH/opioid balance is shifted in either direction the effects of that peptide become salient. Thus, opiate receptor blockade may (i) attenuate opiate analgesia (Acute), (ii) precipitate withdrawal (Tolerant/Dependent) or (iii) reduce the severity of the opiate abstinence syndrome by blocking the unattenuated actions of ACTH (Withdrawn), depending on timing of antagonist administration. See text for details.

TOLERANCE/DEPENDENCE

In the first instance, it is assumed that under baseline conditions in opiate naive animals there is a functional balance between excitatory ACTH influences and β-endorphin mediated inhibition. In behaving organisms, this balance may be tipped in either direction by environmental circumstance. Thus, certain situations may stimulate ACTH release thereby inducing hyperalgesia and increased grooming behavior [6,20] whilst other situations (e.g., uncontrollable noxious stimuli) activate endorphinergic mechanisms such that analgesia becomes the salient reaction [53,54]. Acute opiate administration to a large extent mimics the actions of endogenously released opioids [62]. Thus, potency of analgesic reaction is dependent on the degree of imbalance in the ACTH/opioid ratio, whilst duration is controlled by (i) rate of opiate elimination and (ii) rate of opioid-induced ACTH release. Eventually, the ACTH/opioid ratio equalizes, at which point analgesia is no longer detectable. ACTH levels are then gradually reduced as the opiate continues to be eliminated.

In situations where opiate administration is continued at a constant dosage, tolerance is induced. Essentially, in a chronic drug regimen, each successive administration of opiate acts to maintain ACTH concentrations at excessively high levels. Subsequent doses of opiate fail to induce analgesia as the ACTH/opioid ratio is not significantly shifted toward inhibition. In constant release preparations [78], tolerance occurs when ACTH levels are increased to counterbalance functionally equivalent opiate concentrations. As above, when the ACTH/opioid ratio has balanced, analgesia and other behavioural influences are not detected.

Dependence occurs when each successive dose of opioid is sufficiently greater than the previous treatment. In the same manner as previously described, the opioid induces a corresponding increase in the levels of ACTH. However, the following larger dose of opioid enables a significant shift in the ACTH/opioid ratio to occur such that opioid-induced behavioural changes may be observed. The greater concentrations of opioid introduced into the system in chronic dependence-inducing regimens stimulate the release of more and more ACTH. Eventually, considerable amounts of opioid are required simply to counteract the residual effects of the high levels of ACTH produced as the result of the previous administration of increasing doses of opioid.

Therefore, tolerance and dependence are induced by the same mechanisms. The former is achieved when successive doses of opioid are insufficient to overcome the excitatory effects of increased ACTH activity, whilst the latter ensues when the available concentrations of opioid are significantly greater than previous doses. In both instances, the

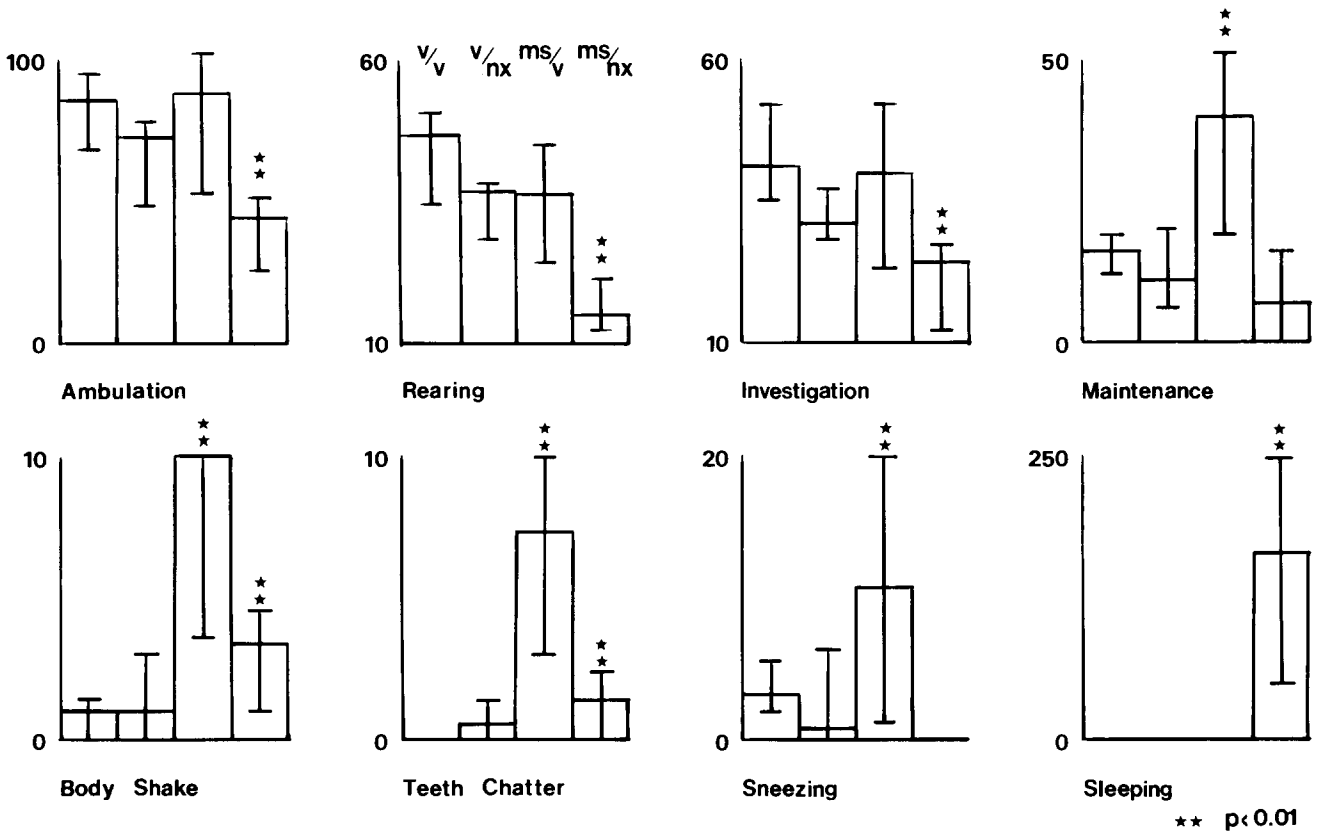

FIG. 2. Effects of abstinence and naloxone (1.5 mg/kg) on behaviours displayed by morphine dependent rats. Frequency data are presented as medians (Lower-Upper quartiles). Statistical analysis revealed significant abstinence-induced exacerbations of maintenance, body shake, teeth chatter and sneezing (MS/V) and their marked attenuation by naloxone (MS/NX). This antagonist further induced significant increases in sleeping time (seconds) and consequently decreases in ambulation, rearing and investigatory behaviours. See text for details.

ACTH/opioid ratio eventually stabilizes but, in the case of dependency, at higher and higher levels.

WITHDRAWAL

Thus, it can be seen that upon cessation of drug regimen opioid elimination continues, producing a shift in the ACTH/opioid ratio towards excitation, with ACTH effects becoming increasingly more pronounced as the level of opioid continues to fall. Therefore, it may be predicted that opiate withdrawal syndrome is mediated by the unattenuated influence of ACTH. This view has been proposed by other workers [7,36] and is consistent with observations that (i) $ACTH_{1-24}$ precipitates withdrawal in morphine dependent rats [7] and (ii) opiate withdrawal is correlated with a significant increase in ACTH levels [50]. In this context it is noteworthy that central injections of $ACTH_{1-24}$ may induce many of the behavioural effects observed during the opiate withdrawal syndrome [36].

Whilst the postulated involvement of ACTH in the mediation of opiate withdrawal may provide an explanation for the mechanisms of the abstinence-induced withdrawal syndrome, it does not account for the phenomenon of naloxone-precipitated withdrawal. The current model may however provide such an explanation. As has been previously stated, ACTH and β-endorphin are maintained in functional equilibrium under normal circumstances, with a shift in either direction rendering the effects of that peptide salient. This balance has two components, ACTH-induced excitation and β-endorphin mediated inhibition. In tolerant and dependent animals, the exogenous opiate acts synergistically with the endogenous opioid and hence the equation is held at a higher level than in drug naive animals. Under these circumstances therefore, behavioural and cellular excitations are held in check by opioid-mediated inhibitory influences. However, upon administration of an opiate antagonist both ACTH and opioid effects are blocked. Thus, in the *absence* of any inhibitory influences withdrawal ensues. Since this antagonist-induced disinhibition is (by contrast to endogenous ACTH-mediated excitation) very rapidly produced, precipitated withdrawal may be expected to be of greater intensity than abstinence-induced withdrawal. Further, the effects of opiate receptor blockade should be directly related to the level at which the ACTH/opioid ratio is held. In support of this suggestion it has been found that the withdrawal syndrome in animals rendered dependent to high doses of morphine is significantly more severe than in rats chronically exposed to lower levels [67].

Therefore, precipiated withdrawal appears to be the result of the antagonism of opioid-induced inhibition, whilst abstinence-induced withdrawal is mediated by the excitatory influences of ACTH acting at opiate receptors. On this view it may be predicted that opiate antagonist administration during primary abstinence should significantly attenuate ACTH

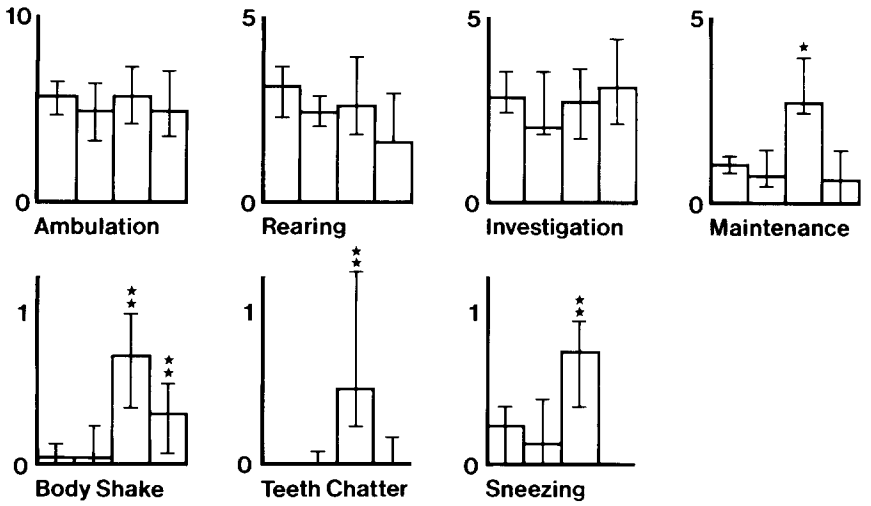

FIG. 3. Effects of abstinence and naloxone (1.5 mg/kg) on behaviours displayed by morphine dependent rats. Analysis excluding periods of somnolence. Data are presented as medians (Lower-Upper quartiles) of mean behavioural responding per minute active. Column (C)1=V/V; C2=V/NX; C3=MS/V; C4=MS/NX. Whilst naloxone had no effect on measures of ambulation, rearing or investigation in post-dependent animals, this antagonist induced marked decreases in maintenance, body shake, teeth chatter and sneezing behaviours. See text for details.

mediated excitation and thereby reduce the severity of the opiate withdrawal syndrome. The present study was conducted in order to investigate this hypothesis.

METHOD

Subjects

Forty adult male Sprague-Dawley rats (350–450 g) from Bradford University Breeding Unit were used. Animals were individually-housed for 3 weeks prior to the start of experimentation (cage size: 45×28×30 cm) and maintained on a 12 hour light/dark cycle (lights on: 0800 hr) in a temperature controlled room (24±1°C). Food and water were available ad lib throughout the study.

Apparatus

Analgesia was assessed by measuring the latency to hind-paw lick in response to hot-plate stimulation, the temperature of which was controlled at 55±1°C. A cut-off of 120 sec was used to prevent tissue damage. Colonic temperature was measured in tube-restrained animals by means of an ITT u23uS thermistor probe inserted 3 cm into the rectum for 20 seconds.

Behaviour was recorded on videotape for subsequent detailed analysis. Only the camera was present in the testing laboratory with ancillary equipment being housed in an adjacent room.

Drugs

Morphine sulphate was dissolved in 0.4 g/ml sweetened milk, solutions of which were freshly prepared twice daily. Naloxone hydrochloride was dissolved in physiological saline (0.9%). All injections were performed intraperitoneally in a volume of 1 ml/kg.

PROCEDURE

Following 3 days habituation to sweetened milk solutions, rats were assigned to drug condition in randomised counterbalanced order, with 20 animals exposed to 0.5 mg/ml morphine sulphate solutions available ad lib for a period of 9 days (mean intake=74.6 ml/day). The remaining 20 animals were exposed to milk alone for the same period (mean intake=98.8 ml/day). At the end of the 9th day both milk and morphine sulphate solutions were withdrawn. Twenty-four hours later, animals were transported to the laboratory, habituated for two hours and randomly assigned to receive either 1.5 mg/kg naloxone hydrochloride or 0.9% saline. In all, four experimental conditions (n=10/group), milk/saline (V/V), milk/naloxone (V/NX), morphine sulphate/saline (MS/V) and morphine sulphate/naloxone (MS/NX) were used. Ten minutes post-injection animals were introduced into an observation cage (45×28×20 cm) and their behaviour recorded on videotape over the next 15 minutes. Immediately following this period, hot-plate tests were carried out, after which temperatures were recorded. The experimenter remained blind to drug condition throughout, with codes only being broken after complete analysis.

Data Analysis

Analysis of behavioural data was performed utilising computer-aided data-logging techniques [28,29] to record frequency (f) and duration (d) of behavioural elements. In view of their non-parametric nature, data for each behavioural element were analysed by Kruskal-Wallis one-way analysis of variance in cases of significance, further comparisons were performed using Mann-Whitney tests.

Hot-plate latency data were initially subjected to a two-factor analysis of variance (ANOVA: Factor A, chronic milk or morphine; Factor B, acute saline or naloxone). Further

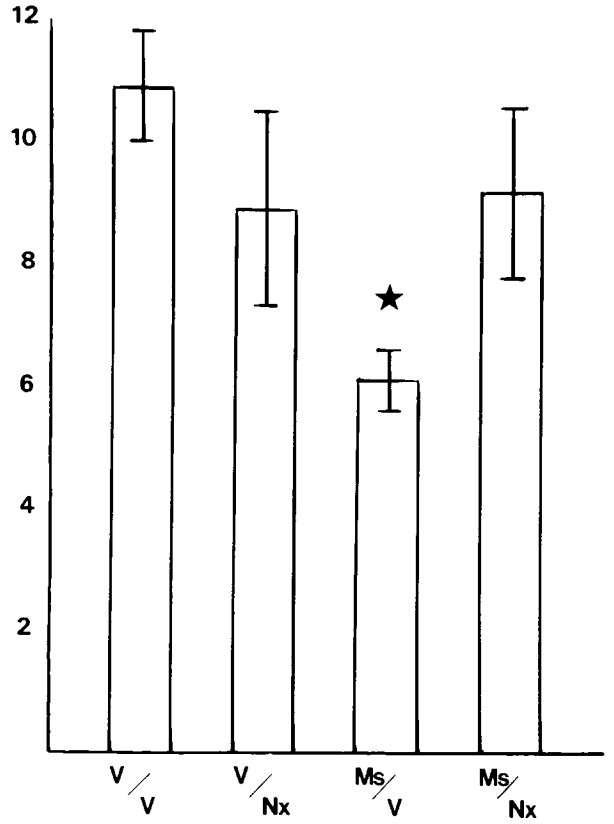

FIG. 4. Effects of morphine abstinence and naloxone on hot-plate latencies. Data are expressed as means (±SEM). Statistical analysis revealed significant abstinence-induced hyperalgesia (MS/V) and its complete attenuation by naloxone (MS/NX). *$p<0.05$.

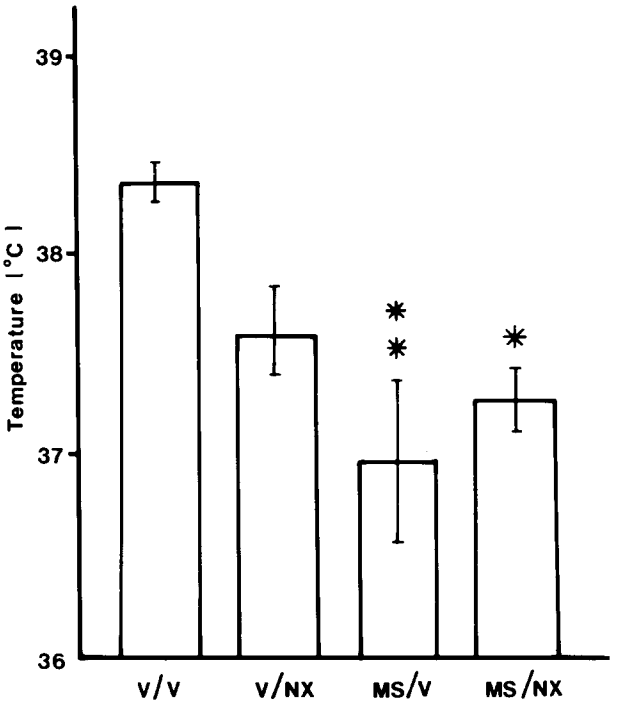

FIG. 5. Effects of morphine abstinence and naloxone on rectal temperature. Data are presented as means (±SEM). Statistical analysis revealed significant abstinence-induced hypothermia (MS/V) and the lack of effect of naloxone in antagonizing this response (MS/NX). *$p<0.05$, **$p<0.01$.

analytical comparisons, which were required in view of significant interactions, were performed using Dunnett's procedure for comparing all treatments with control. Temperature data were also subjected to 2-factor ANOVA and subsequent Dunnett's tests.

RESULTS

Behavioural data are summarized in Fig. 2. Results indicate firstly, a significantly greater incidence of maintenance grooming, body shaking, teeth chattering and sternutation in morphine sulphate/saline (MS/V) treated animals as compared with milk/saline (V/V) controls, data which strongly suggest the successful induction of dependence. Secondly, as the only measure altered in the milk/naloxone (V/NX) condition was a slight, but significant, decrease in investigatory behaviour, naloxone would appear to exert very little influence in non-dependent rats. Thirdly, and in direct contrast to the above, naloxone induced marked changes in the behaviour of post-dependent animals, with significant attenuation of abstinence-exacerbated maintenance, body shaking, teeth chattering and sneezing behaviours being coupled with a considerable increase in sleeping time ($p<0.01$). As a consequence of the latter effect, measures of ambulation, rearing and investigation were all significantly reduced ($p<0.01$). Finally, whilst maintenance behaviours and sneezing were not appreciably different from milk/saline controls, body shaking and teeth chattering behaviours in the morphine sulphate/naloxone (MS/NX) treatment group were increased ($p<0.01$). Importantly, however, the incidence of both body shaking and teeth chattering in these animals were significantly lower ($p<0.01$) than those observed in the morphine sulphate/saline condition.

In view of the marked increase in sleeping time observed in the morphine sulphate/naloxone treatment condition, it was possible that the apparent antagonism of the morphine withdrawal syndrome was produced as the result of a previously unreported sedative action of naloxone under these conditions. Although decreases in body tone and reactivity (consistent with this hypothesis) were not observed, data were re-analysed excluding periods of somnolence. Standardised behavioural data, expressed as mean behavioural responding per minute active, are presented in Fig. 3. This analysis revealed that whilst naloxone had no significant influence on measures of ambulation, rearing or investigation, marked decreases (as compared to morphine sulphate/saline control) in abstinence-induced behavioural exacerbations were still observed. These data are indicative of a significant naloxone-induced attenuation of behaviours usually associated with the morphine abstinence syndrome.

Hot-plate latency data are summarized in Fig. 4. ANOVA revealed a significant chronic-× acute-treatment interaction, $F(1,36)=4.4$, $p<0.05$, which further detailed analysis indicated to be due to significant reductions in pain latencies under MS/V, $t(36)=2.82$, $p<0.05$. These data demonstrate profound abstinence-induced hyperalgesia (MS/V) and, as

V/V and MS/NX were not significantly different, $t(36)=1.18$, ns, its complete attenuation by 1.5 mg/kg naloxone.

Temperature data are summarized in Fig. 5. ANOVA applied to these data also indicated a significant chronic-× acute-treatment interaction, $F(1,36)=4.21$, $p<0.05$, which follow-up tests revealed to be due to an influence of differences between V/V-MS/V, $t(36)=3.77$, $p<0.01$, and V/V-MS/NX, $t(36)=2.95$, $p<0.05$. These data are indicative of naloxone-insensitive hypothermia during primary abstinence.

DISCUSSION

Current data confirm that oral self-administration is an effective method for the induction of morphine dependence in rats [41], as indicated by observed behavioural [12], nociceptive [67,76] and thermic [44] responses during withdrawal. Further, in the absence of major effects in non-dependent animals, naloxone markedly attenuated all abstinence-induced behavioural exacerbation whilst significantly increasing sleeping time. Measures of ambulation, rearing and investigation were not significantly different from milk/saline controls when data were re-analysed to allow for the possible confounding influence of this reaction. Therefore, the observed increase in sleeping time does not account for the pronounced antagonism of abstinence-induced increases in grooming, body shaking, teeth-chattering and sneezing produced by naloxone in post-dependent animals. Finally, withdrawal-induced hypothermia was not influenced by naloxone administration, perhaps indicating its greater resistance to attenuation by opiate antagonists or its mediation by non-opioid mechanisms. By contrast however, naloxone completely attenuated abstinence-induced hyperalgesia, a finding entirely consistent with previous demonstrations of naloxone-sensitive environmentally-induced hyperalgesia [30,53]. Together, these data suggest a significant involvement of opioidergic mechanisms in the mediation of abstinence-induced behavioural exacerbations and withdrawal hyperalgesia.

Patently, current findings are at variance with the wealth of literature concerning naloxone-precipitated withdrawal. Whilst it is possible that this compound has agonist-like properties in post-dependent animals, this interpretation would seem unlikely in view of evidence indicating naloxone to have relatively pure antagonist actions in rats at doses of less than 5 mg/kg [58]. However, although more extensive experimentation is needed to further clarify this point, it is believed that the present discrepancy may relate to timing of antagonist administration. On the basis of the current model it is envisaged that naloxone in dependent animals would block the actions of both opioids and ACTH. Withdrawal is therefore the result of naloxone-induced disinhibition. Conversely, in abstinent animals where it is postulated that withdrawal is mediated by the unattenuated excitatory actions of ACTH, naloxone antagonises these effects. Inhibition is thereby reinstated and a marked reduction in the severity of the morphine withdrawal syndrome ensues.

Whilst firm conclusions concerning the role of ACTH in the mediation of opiate withdrawal cannot be made on the basis of present data, it is noteworthy that in addition to the in vitro and in vivo evidence suggesting a role for ACTH as an endogenous contra-opioid agonist, many of the biochemical correlates of withdrawal are also consistent with the proposed involvement of ACTH in this phenomenon. Acute administration of opiates decrease intracellular concentrations of cAMP and CA^{2+}, whilst chronic exposure increases these levels. In abstinent systems both cAMP and cytosol Ca^{2+} concentrations are raised to supranormal levels. Thus, it has been postulated that these changes in the adenylate cyclase-cAMP "second messenger" system (for review see [11]) and Ca^{2+} (for review see [79]) may reflect cellular hyperirritability that is more usually inhibited by the actions of opioids. However, as ACTH stimulates adenylate cyclase and cAMP activity in a calcium dependent manner [19, 57, 83, 84] situations where ACTH activity is increased should be correlated with raised intracellular concentrations of adenylate cyclase, cAMP and Ca^{2+}. Thus, the increases in these cellular parameters observed during opiate withdrawal are in accordance with the proposed mediation of this syndrome by the disinhibitory influences of opiate antagonists or the excitatory actions of ACTH. Interestingly, brain homogenates of post-dependent rats have been found to contain a peptide fraction which specifically inhibits morphine analgesia [71] thereby further suggesting that opiate withdrawal is correlated with the *presence* of an endogenous contra-opioid agonist rather than the *absence* of opioids *per se*.

Thus, in conclusion, a model of opiate tolerance and dependence is proposed whereby withdrawal is a consequence of the disruption of a functional balance held between ACTH-excitatory and opioid-mediated inhibitory influences. Data indicating an ability of naloxone to markedly attenuate abstinence-induced behavioural exacerbations are consistent with this view and the hypothesis first proposed by Tatum *et al.* [63] that "Addiction . . . is largely a question of physiological balance between stimulation and depression at any given level of irritability . . ." (p. 473). Whilst the current model must, in the absence of further experimental data, remain speculative it may prove to have heuristic value in the further development of our understanding of the mechanisms of opiate tolerance, dependence and withdrawal.

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REFERENCES

1. Akil, H., W. A. Hewlett, J. D. Barchas and C. H. Li. Binding of 3H-β-endorphin to rat brain membraines: characterization of opiate properties and interaction with ACTH. *Eur J Pharmacol* **64:** 1–8, 1980.
2. Amir, S. Effects of ACTH on pain responsiveness in mice: interaction with morphine. *Neuropharmacology* **20:** 959–962, 1981.
3. Belcher, G., T. Smock and H. L. Fields. Effects of intrathelal ACTH on opiate analgesia in the rat. *Brain Res* **247:** 373–377, 1982.
4. Bertolini, A., W. Ferrari, W. Fratta, G. L. Gessa, G. Mereu and A. Tagliamonte. Sexual arousal in male animals: a central effect of ACTH-like peptides in mammals. In: *Peptides: Chemistry, Stuctures and Biology*, edited by W. Roderich and J. Meienhoffer. Ann Arbor: Ann Arbor Science Publishers Inc., 1975, pp. 659–665.

5. Bertolini, A., G. L. Gessa and W. Ferrari. Penile erection and ejaculation: a central effect of ACTH-like peptides in mammals. In: *Sexual Behaviour: Pharmacology and Biochemistry*, edited by M. Sandler and G. L. Gessa. New York: Raven Press, 1975, pp. 247–257.
6. Bertolini, A., R. Poggioli and W. Fratta. ACTH-induced hyperalgesia in rats. *Experientia* **35:** 1216–1217, 1978.
7. Bertolini, A., R. Poggioli and W. Fratta. Withdrawal symptoms in morphine-dependent rats intracerebroventricularly injected with $ACTH_{1-24}$ and β-MSH. *Life Sci* **29:** 249–252, 1981.
8. Bhargava, H. N. The effects of naturally occuring and D-Amino acid containing enkaphalins on morphine abstinence and locomotor activity. In: *Characteristics and Function of Opioids*, edited by J. M. Van Ree and L. Terenius. Amsterdam: Elsevier/North Holland, 1978, pp. 53–56.
9. Bradbury, A., D. Smyth, C. Snell, J. Deakin and S. Wendlant. Comparison of the analgesic properties of lipotropin C-fragment and stabilized enkephalins in the rat. *Biochem Biophys Res Commun* **74:** 748–754, 1977.
10. Cochin, J. and B. E. Mushlin. Effect of agonist-antagonist interaction on the development of tolerance and dependence. *Ann NY Acad Sci* **281:** 244–251, 1976.
11. Collier, H. O. J. Cellular aspects of opioid tolerance and dependence. In: *Opioids Past, Present and Future*, edited by J. Hughes, H. O. J. Collier, M. J. Rance and M. B. Tyers. London: Taylor and Francis, 1984, pp. 109–125.
12. Collier, H. O. J., D. L. Francis and C. Schneider. Modification of morphine withdrawal by drugs interacting with humoral mechanisms: some contradictions and their interpretation. *Nature* **237:** 220–223, 1972.
13. Collier, H. O. J. and A. C. Roy. Morphine-like drugs inhibit the stimulation by E prostaglandins of cyclic AMP formation by rat brain homogenate. *Nature* **248:** 24–27, 1974.
14. Cox, B. M., K. E. Opheim, H. Teschemacher and A. Goldstein. A peptide-like substance from pituitary that acts like morphine. *Life Sci* **16:** 1777–1782, 1975.
15. Crain, S. M., B. Crain, T. Finnigan and E. J. Simon. Development of tolerance to opiates and opioid peptides in organotypic cultures of mice spinal cord. *Life Sci* **25:** 1797–1802, 1979.
16. Ferri, S., D. Cocchi, V. Locatelli, S. Spampinato and E. Muller. $ACTH_{1-24}$ counteracts the prolactin-releasing effect of an opioid. *Eur J Pharmacol* **77:** 143–145, 1982.
17. Fraioli, F., C. Moretti, D. Paolucci, E. Alicicco, F. Crescenzi and G. Fortunio. Physical exercise stimulates marked concomitant release of beta-endorphin and adrenocorticorropic hormone (ACTH) in peripheral blood in man. *Experientia* **36:** 987–989, 1980.
18. Fratna, W., Z. L. Rossetti, R. Poggioli and G. L. Gessa. Reciprocal antagonism between $ACTH_{1-24}$ and β-endorphin in rats. *Neurosci Lett* **24:** 71–74, 1981.
19. Gill, G. N. Mechanism of ACTH action. *Metabolism* **21:** 571–588, 1972.
20. Gispen, W. H. and R. L. Isaacson. ACTH-induced excessive grooming in the rat. *Pharmacol Ther* **12:** 209–246, 1981.
21. Gispen, W. H. and W. M. Wiegant. Opiate antagonists suppress $ACTH_{1-24}$ induced excessive grooming in the rat. *Neurosci Lett* **2:** 159–164, 1976.
22. Gold, M. S., R. Byck, D. R. Sweeney and H. D. Kleber. Endorphin-Locus Colroleus connection mediates opiates action and withdrawal. *Biomedicine* **30:** 1–4, 1979.
23. Gold, M. S., C. A. Dackis, A. L. C. Pottash, H. H. Sternbach, W. J. Annitto, D. Martin and M. Dackis. Naltrexone, opiate addiction, and endorphins. *Med Res Rev* **2:** 211–246, 1982.
24. Griswell, H. E. and M. David. Partial Antagonism of Stress-induced analgesia by ACTH pretreatment. *Soc Neurosci Abstr* **5:** 1768, 1979.
25. Guillemin, R., T. M. Vargo, J. Rossier, S. Minick, N. Ling, C. Rivier, W. Vale and F. Bloom. β-endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. *Science* **197:** 1367–1369, 1977.
26. Havemann, U. and K. Kushinsky. Opiates inhibit prostaglandin E_2-stimulated adenylcyclase in skin of corpus striatum of rats. In: *Characteristics and Function of Opioids*, edited by J. M. Van Ree and L. Terenius. Amsterdam: Elsevier, 1978, pp. 355–356.
27. Hendrie, C. A. Naloxone antagonism of the morphine withdrawal syndrome. *Br J Pharmacol* **84:** 51p, 1985.
28. Hendrie, C. A. and S. Bennett. A microcomputer technique for the detailed analysis of animal behaviour. *Physiol Behav* **30:** 233–235, 1983.
29. Hendrie, C. A. and S. Bennett. A microcomputer technique for the detailed behavioural, and automatic statistical analysis of animal behaviour. *Physiol Behav* **32:** 865–869, 1984.
30. Hendrie, C. A. and R. J. Rodgers. Naloxone-reversible hyperalgesia in female rats following copulatory experience. *Br J Pharmacol* **76:** 199p, 1982.
31. Ho, W. K., H. L. Wen and N. Ling. β-endorphin-like immunoactivity in the plasma of heroin addicts and normal subjects. *Neuropharmacology* **19:** 117–120, 1980.
32. Hosobuchi, Y., M. Meglio, J. E. Adams and C. H. Li. Beta-endorphin: development of tolerance and its reversal by 5-hydroxytryptophan in cats. *Proc Natl Acad Sci USA* **74:** 4017–4019, 1977.
33. Hughes, J. Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. *Brain Res* **88:** 295–308, 1975.
34. Huidobro-Toro, J. P. and E. L. Way. Studies on tolerance development to β-endorphin. In: *Characteristics and Functions of Opioids*, edited by J. N. van Ree and L. Terenius. Amsterdam: Elsevier/North Holland, 1978, pp. 61–62.
35. Huidobro-Toro, J. P. and E. L. Way. Rapid development of tolerance in the hyperthermic effect of β-endorphin, and cross-tolerance between enkephalins and beta-endorphin. *Eur J Pharmacol* **65:** 221–231, 1980.
36. Jacquet, Y. F. Opiate effects after adrenocorticotropin or β-endorphin injection in the periaqueductal gray matter of rats. *Science* **201:** 1032–1034, 1978.
37. Jezova, D., M. Vigas and S. Jurcovicova. ACTH and corticosterone response to naloxone and morphine in normal, hypophysectomized and Dexamethasone-treated rats. *Life Sci* **31:** 307–314, 1982.
38. Jolles, J., V. M. Wiegant and W. H. Gispen. Reduced behavioural effectiveness of $ACTH_{1-24}$ after a second administration: interactions with opiates. *Neurosci Lett* **9:** 261–266, 1978.
39. Kosterlitz, H. W. and J. Hughes. Possible physiological significance of enkephalin, an endogenous ligand of opiate receptors. In: *Advances in Pain Research and Therapy*, vol 1, edited by J. J. Bonica and D. Albe-Fessard. New York: Raven Press, 1976, pp. 641–654.
40. Krivoy, W. A., D. C. Kroeger and E. Zimmerman. Neuropeptide influence on acute and chronic effects of opiates. *Psychoneuroendocrinology* **2:** 43, 1977.
41. Kumar, R., H. Steinberg and I. P. Stolerman. How rats can become dependent on morphine in the course of relieving another need. In: *Scientific Basis of Drug Dependence*, edited by H. Steinberg. London: Churchill, 1969, pp. 209–220.
42. Lis, M., N. Lariviere, G. Maurice, J. Julesz, N. Seidah and M. Chretien. Concomitant changes of both ACTH, β-endorphin and N-terminal portion of pro-opiomelanocortin in rats. *Life Sci* **30:** 1159–1164, 1982.
43. Lotti, V. S., N. Kokka and R. George. Pituitary-adrenal activation following intrahypothalamic microinjection of morphine. *Neuroendocrinology* **4:** 326–332, 1969.
44. Martin, W. R. Pharmacology of opioids. *Pharmacol Rev* **35:** 283–323, 1984.
45. Mello, N. K. and J. H. Mendelson. Self-administration of an enkephalin analog by rhesus monkey. *Pharmacol Biochem Behav* **9:** 579–586, 1978.
46. Meyerson, B. J. Comparison of the effects of β-endorphin and morphine on exploratory and socio-sexual behaviour in the male rat. *Eur J Pharmacol* **69:** 453–463, 1981.

47. Meyerson, B. J. and L. Terenius. β-endorphin and male sexual behaviour. *Eur J Pharmacol* **42:** 191–192, 1977.
48. Morley, J. E. Synthetic endorphins. In: *Perspectives in Peptide Chemistry*, edited by A. Eberle, R. Geiger and J. Wiezand. Basel: J. Karger, 1981, pp. 329–343.
49. Motomatsu, T. M., M. Lis, N. Seidah and M. Chretien. Inhibition by beta-endorphin of dopamine-sensitive adenylate cyclase in rat striatum. *Biochem Biophy Res Commun* **77:** 442–447, 1977.
50. Munson, P. L. Effects of morphine and related drugs on the corticotrophin (ACTH)-stress reaction. *Prog Brain Res* **39:** 361–372, 1973.
51. O'Donohue, T. L. and D. M. Dorsa. The opiomelanotropinergic neuronal and endocrine systems. *Peptides* **3:** 353–395, 1982.
52. Rivier, C., M. Brownstein, J. Spiess, J. Rivier and W. Vale. In vivo corticotropin-releasing factor-induced secretion of adrenocorticotropin, β-endorphin and corticosterone. *Endocrinology* **110:** 272–278, 1982.
53. Rodgers, R. J. and C. A. Hendrie. Social conflict activates status-dependent endogenous analgesic or hyperalgesic mechanisms in male mice: effects of naloxone on nociception and behaviour. *Physiol Behav* **30:** 775–780, 1983.
54. Rodgers, R. J., C. A. Hendrie and A. J. Waters. Naloxone partially antagonizes post-encounter analgesia and enhances defensive responding in male rats exposed to attack from lactating female conspecifics. *Physiol Behav* **30:** 781–786, 1983.
55. Roemer, D., H. Buescher, R. Hill, J. Pless, W. Bauer, F. Cardinaux, A. Closse, d. Hauser and R. Huquenin. A synthetic enkaphalin analogue with prolonged parenteral and oral analgesic activity. *Nature* **268:** 547–549, 1977.
56. Rossier, J., E. French, C. Gros, S. Minick, R. Guillemin and F. E. Bloom. Adrenalectomy, dexamethasone or stress alters opioid peptide levels in rat anterior pituitary but not intermediate like on brain. *Life Sci* **25:** 2105, 1979.
57. Rudman, D. and J. W. Isaacs. Effect of intratheral injection of melanotropic-lipolytic peptides on the concentration of 3′, 5′-cyclic adenosine monophosphate in cerebrospinal fluid. *Endocrinology* **97:** 1476–1485, 1975.
58. Sawynok, J., C. Pinsky and F. S. Labella. On the specificity of naloxone as an opiate antagonist. *Life Sci* **25:** 1621–1632, 1979.
59. Stefano, G. B., E. J. Catapane and R. M. Kream. Characterization of the dopamine stimulated adenylate cyclase in the pedal ganglia of mytilus edulis: interactions with etorphine β-endorphin, DALA and methionine enkephalin. *Cell Mol Neurobiol* **1:** 57–68, 1981.
60. Stevens, D. R. and W. R. Klemm. Morphine-naloxone interactions: A role for nonspecific morphine excitatory effects in withdrawal. *Science* **205:** 1379–1380, 1979.
61. Sunantoo, R. Glucocorticoids inhibit endorphin synthesis by pituitary cells. *Nature* **280:** 684–685, 1979.
62. Szara, S. Opiate receptors and endogenous opiates: Panorama of opiate research. *Prog Neuropsychopharmacol Biol Psychiatry* **6:** 3–15, 1982.
63. Tatum, A. L., M. H. Seevers and K. H. Collins. Morphine addiction and its physiological interpretation based on experimental evidences. *J Pharmacol Exp Ther* **36:** 447–475, 1929.
64. Terenius, L. Effect of peptides and aminoacids on dihydromorphine binding to the opiate receptor. *J Pharm Pharmacol* **27:** 450–452, 1975.
65. Terenius, L. Somatostatin and ACTH are peptides with partial antagonist-like selectivity for opiate receptors. *Eur J Pharmacol* **38:** 211–213, 1976.
66. Terenius, L., W. H. Gispen and D. De Wied. ACTH-like peptides and opiate receptors in the rat brain: structure-activity studies. *Eur J Pharmacol* **33:** 395–399, 1975.
67. Tilson, H. A., R. H. Rech and S. Stolman. Hyperalgesia during withdrawal as a means of measuring the degree of dependence on morphine dependent rats. *Psychopharmacologia (Berlin)* **28:** 287–300, 1973.
68. Tseng, L.-F., H. H. Loh and C. H. Li. β-endorphin as a potent analgesic by intravenous injection. *Nature* **263:** 239, 1976.
69. Tseng, L.-F., H. Loh and C. H. Li. Human β-endorphin: Development of tolerance and behavioural activity in rats. *Biochem Biophys Res Commun* **74:** 390–396, 1977.
70. Tseng, L.-F., E. T. Wei, H. H. Loh and C. H. Li. Beta-endorphin: central sites of analgesia, catalepsy and body temperature changes in rats. *J Pharmacol Exp Ther* **214:** 328–332, 1980.
71. Ungar, G., A. L. Ungar, D. H. Malin and D. Sarantakis. Brain peptides with opiate antagonist action: Their possible role in tolerance and dependence. *Psychoneuroendocrinology* **2:** 1–10, 1977.
72. Van Loon, G. R. and E. B. Souza. Development of tolerance to the ACTH releasing effects of beta-endorphin. *Res Commun Chem Pathol Pharmacol* **22:** 203–204, 1978.
73. Van Ree, J. M., D. G. Smyth and F. C. Colpaert. Dependability creating properties of lipotropin C-fragment (beta-endorphin): Evidence for its internal control of behaviour. *Life Sci* **24:** 495–502, 1979.
74. Van Ree, J., D. de Wied, A. Bradbury, A. Hulme, D. Smyth and C. Snell. Induction of tolerance to the analgesic action of lipotropin C-fragment. *Nature* **264:** 792–793, 1976.
75. Van Vugt, D. A. and J. Meites. Influence of endogenous opiates on anterior pituitary function. *Fed Proc* **39:** 2533–2538, 1980.
76. Vonvoitlander, P. F. and R. A. Lewis. A withdrawal hyperalgesia test for physical dependence: evaluation of μ and mixed partial opioid agonists. *J Pharmacol Meth* **10:** 277–282, 1983.
77. Waterfield, A., J. Hughes and H. W. Kosterlitz. Cross-tolerance between morphine and methionine-enkaphalin. *Nature* **260:** 624–625, 1976.
78. Way, E. L., H. H. Loh and F. H. Shen. Simultaneous quantitative assessment of morphine tolerance and physical dependence. *J Pharmacol Exp Ther* **167:** 1–8, 1969.
79. Way, E. L. and A. Rezvani. Opiate tolerance and physical dependence: assessment and mechanisms. In: *Opioids Past, Present and Future*, edited by J. Hughes, H. O. J. Collier, M. J. Rance and M. B. Tyers. London: Taylor and Francis, 1984, pp. 103–108. 1984, pp. 103–108.
80. Wei, E. Enkephalin Analogs: Correlation of Potencies for analgesia and physical dependence. In: *Characteristics and Function of Opioids*, edited by J. M. Van Ree and L. Terenius. Amsterdam: Elsevier/North Holland, 1978, pp. 445–446.
81. Wei, E. and H. Loh. Physical dependence and opiate-like peptides. *Science* **193:** 1262–1263, 1976.
82. Wei, E., H. H. Loh and E. L. Way. Quantitative aspects of precipitated abstinence in morphine dependent rats. *J Pharmacol Exp Ther* **184:** 398–408, 1973.
83. Wiegant, V. M., A. J. Dunn, P. Schotman and W. H. Gispen. ACTH-like neurotropic peptides: possible repilators of rat brain cyclic AMP. *Brain Res* **168:** 565–584, 1979.
84. Wiegant, V. M. and W. H. Gispen. Behaviourally-active ACTH analogues and brain cyclic AMP. *Exp Brain Res* **23:** Suppl 219, 1975.
85. Wiegant, W. M., W. H. Gispen, L. Terenius and D. De Wied. ACTH-like peptides and morphine: interaction at the level of the CNS. *Psychoneuroendocrinology* **2:** 63–69, 1976.
86. Wiegant, V. M., J. Jolles and W. H. Gispen. β-endorphin grooming in the rat: single dose tolerance. In: *Characteristics and Function of Opioids* edited by J. M. Van Ree and L. Terenius. Amsterdam: Elsevier/North Holland, 1978, pp. 447–450.
87. Wiley, J. N. and D. A. Downs. Naloxone-precipitated jumping in mice pretreated with acute injections of opioids. *Life Sci* **25:** 797–801, 1979.
88. Wollemann, M. Endogenous opioids and cyclic AMP. *Prog Neurobiol* **16:** 145–154, 1981.