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STRESS-INDUCED RELEASE OF BRAIN AND PITUITARY β-ENDORPHIN: MAJOR ROLE OF ENDORPHINS IN GENERATION OF HYPERTHERMIA, NOT ANALGESIA

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SUMMARY

The present paper examines the conjectured causal relationship between the alterations in brain, pituitary and plasma levels of endorphins and the antinociception (analgesia) and hyperthermia elicited by acute stress. A 5-min foot-shock instigated a significant depression in the levels of β-endorphin immunoreactivity (β-EI) in both the hypothalamus and periventricular β-endorphinergic fibre-containing tissue. A large elevation in plasma levels of β-EI, consisting of about 70% β-endorphin (β-EP), and 30% β-lipotropin (β-LPH) was associated with a significant reduction in the β-EI content of both the anterior (AL) and neurointermediate (NIL) lobes of the pituitary. No concomitant changes in the levels of Met-enkephalin immunoreactivity (M-EI) in discrete areas of brain and pituitary were detectable. Application of a high (10 mg/kg) but not a low (1 mg/kg) dose of naloxone, prior to foot-shock, slightly reduced the increase in tail-flick latency evoked by this stress. In contrast, both of these doses strongly and dose-dependently attenuated the accompanying rise in core temperature (Tc). Chronic (~ 30 day) morphine treatment resulted in a 45% decrease in the NIL content of β-EI and a clear depression in its basal plasma levels, although a substantial post-stress rise in plasma β-EI was still found: stress-induced analgesia (SIA) was enhanced, but the concurrent stress-induced hyperthermia (SIH), reduced in morphinized animals. These data demonstrate that stress produces a generalized mobilization of both central and pituitary pools of β-EI, and indicate that endorphins may play a more important role in the mediation of changes in Tc than in the generation of the concomitant increase in nociceptive threshold, upon activation by stress.

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INTRODUCTION

Notwithstanding the considerable effort directed towards the elucidation of the physiological role of endorphins, no specific function(s) can, as yet, be unambiguously ascribed to any of these peptides. The possibility of an activation of endorphinergic systems under stress has frequently attracted attention and stress both elevates plasma levels[18,44], and decreases the hypothalamic content of β-endorphin immunoreactivity (β-EI)[44]. The imposition of stress also generates an analgesia or, more precisely, an increase in nociceptive threshold, antagonizable with a variable degree of efficacy by naloxone[2,11] (see also ref. 33). In addition, a rise in core temperature (Tc) stereospecifically attenuated by naloxone[7] is observed. Further, both analgesia[54] and hyperthermia[6,20,22,25,29,30,46] are inducible in a naloxone-reversible fashion by application of opioid alkaloids or peptides and hyperthermia by low doses relative to those required for analgesia and hypothermia[20,22,25]. However, the unification of these data in a demonstration that a particular stress is effective in both releasing endorphins and producing analgesia and hyperthermia has not, as yet, been achieved.

Thus the present study constitutes an investigation of the naloxone-sensitivity and cross-tolerance to morphine of the analgesia and hyperthermia evoked by a stress model herein characterized as mobilizing both central and pituitary pools of β-EI.

The present data, are suggestive that endorphins' function, upon activation by stress, primarily in the modulation of Tc rather than nociceptive threshold.

A preliminary account of part of these data has been published elsewhere[42].

METHODS

Foot-shock' stress

Male Sprague–Dawley rats (200 g unless otherwise stated), housed in groups of 4, with free access to chow and water, were allowed 4 days acclimatization to laboratory conditions prior to behavioural testing. Environmental temperature was maintained at 23 $\pm$ 1 °C.

Rats were individually subjected to 5 min scrambled, inescapable, intermittent foot-shock with 30 pulses (each of 3 mA and 300 msec duration) per minute applied to the feet.

Nociceptive testing and measurement of core temperature

The tail-flick test was used for evaluation of nociceptive threshold. A focussed light beam was directed at the tip of the tail of gently restrained rats and latency to tail withdrawal electronically recorded. Beam intensity was adjusted to give basal thresholds, read immediately prior to stress or injection, of 3–4 sec. A cut-off of 7.5 sec was observed in order to preclude tissue damage. At each session, 6 latency measurements, successive readings being separated by an interval of 10 sec, were taken, and the mean calculated. The mean latency after stress and/or injection was expressed as a percentage of mean basal levels. A blind design was employed for determination of the influence of naloxone upon stress-induced analgesia (SIA) with the injector/tail-flick

recorder unaware of the identity of the solution (naloxone hydrochloride or saline i.p.) provided. Details are given in figure legends.

Measurement of core temperature was performed by insertion of a thermistor probe into the rectum to a depth of 5 cm, for 30 sec. Stress-induced hyperthermia (SIH) was evaluated as follows: basal Tc was determined, saline or naloxone injected (i.p.), the animal returned to home cage for 5 min, removed, foot-shocked and Tc monitored at the times indicated in the figures.

Development of tolerance to morphine

Rats, of an initial weight 180 g, were rendered tolerant/dependent to morphine by subcutaneous implantation of pellets, each containing 75 mg morphine base, according to the protocol described in Przewłocki et al.[40]. The vocalization tail-root stimulation test (as described in ref. 6) was applied on day 35 of implantation for quantification of the degree of tolerance to morphine of both placebo- and morphine-treated rats. Independent (experimentally naive) groups of rats were employed for each particular study and, thereafter, 'discarded'. For organization of testing, see legends.

Estimation of brain, pituitary and plasma levels of β-EI and Met-enkephalin immunoreactivity (M-EI)

Brain and pituitary. Rats were removed from home cages, decapitated within 10 sec, or foot-shocked and sacrificed 10 min thereafter. Procedure for dissection, extraction and radioimmunoassay (RIA) of levels of β-EI have been published elsewhere[24,40]. The 'structure' termed PVA was composed of the midline nuclear zone of the diencephalon and the central periventricular portion of the mesencephalon, areas wherein a dense population of β-endorphinergic fibres has been visualized.

Plasma. Rats were decapitated either directly after removal from home cages, or at various times after release from foot-shock, with intervening periods passed in rest boxes. Trunk blood was collected and levels of β-EI estimated by RIA (as described in ref. 24).

The β-endorphin (β-EP) antiserum used recognized rat β-EP and β-lipotropin (β-LPH) to an equimolar degree and with a very high avidity (detection limit 5 fmol/tube or 20 fmol/ml plasma), but cross-reacted to α- and γ-endorphin and enkephalins to a negligible extent. Thus β-EI, as presented in the text, is jointly constituted of β-EP and β-LPH. The Met-enkephalin antiserum displayed 0.25% cross-reactivity to Leu-enkephalin and negligible recognition of α-, β- and γ-endorphin or β-LPH. The detection limit was about 50 fmol/tube.

In an attempt to differentiate the particular components of β-EI estimated, gel filtration (Sephadex G-50 column) of fresh, unfrozen plasma pooled from foot-shock-stressed animals and immediately applied to the column was performed as detailed elsewhere[24,41].

Statistical analyses

All statistical analyses were performed by means of the two-tailed Student's *t*-test.

RESULTS

The influence of foot-shock stress upon brain, pituitary and plasma levels of β-EI and M-EI

The stress enforced in the present study (5 min foot-shock) was of short duration and produced negligible (about 2%) weight loss (as estimated 15 min post-stress) with no gross behavioural abnormalities (e.g. immobilization) apparent upon open-field observation post-stress. However, as illustrated in Figs. 1 and 2, this stress was highly effective in activating both brain and pituitary systems of β-EI.

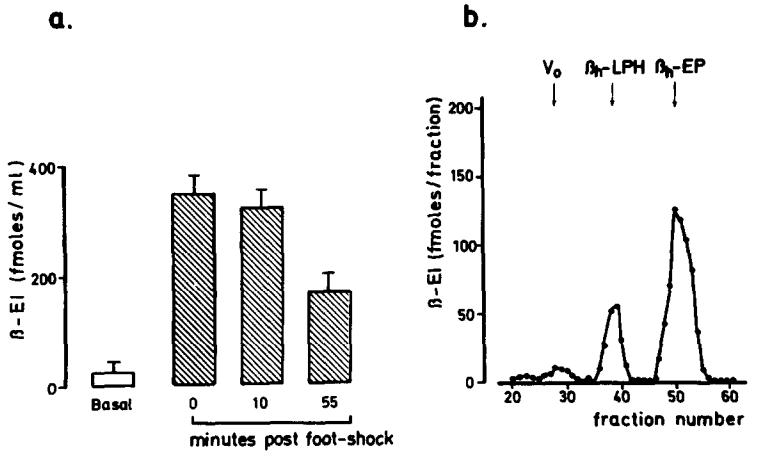

Fig. 1. a: the influence of 5 min foot-shock upon plasma levels of β-endorphin immunoreactivity at various times post-stress. Basal signifies non-stressed. Bars indicate S.E.M., n = 5 per column. Significance of basal vs stressed differences at all times was $P \leqslant 0.001$. b: gel filtration separation of components of plasma β-endorphin immunoreactivity. Four rats were killed 10 min post-stress, their blood immediately centrifuged, plasma taken, pooled (300 μl from each) and applied to the column (Sephadex G-50). Arrows indicate void volume (V_0) and elution volumes of β_h-LPH and β_h-EP.

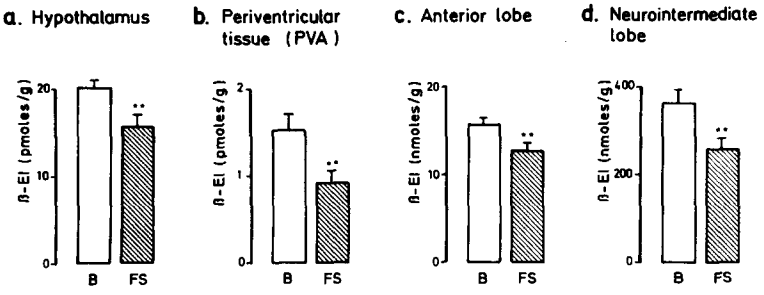

Fig. 2. The influence of 5 min foot-shock upon levels of β-endorphin immunoreactivity in: a, hypothalamus; b, periventricular tissue; c, anterior and d, neurointermediate lobes of the pituitary. B signifies non-stressed and FS, 10 min post foot-shock. Bars indicate S.E.M. Results are the mean of 2 (a and b) or 3 (c and d) separate experiments. Total n: 10, 10, 18 and 18 for a, b, c and d, respectively. Significance of basal vs stressed differences was, in all cases, $P \leqslant 0.01$.

Fig. 1a depicts the large elevation in plasma levels of β-EI produced, which rose from a value close to the detection limit of the assay system to a maximum of about 350 fmol/ml immediately post-stress ($P < 0.001$) and which remained significantly ($P < 0.001$) in excess of basal values, even 55 min post-shock. Gel-filtration analysis of fresh, unfrozen plasma (pooled from 4 animals killed 10 min post-stress) revealed, as shown in Fig. 1b, two peaks, one of which corresponded to human β-EP and the other to human β-LPH, these components being present in the proportions of about 70% and 30%, respectively. (This pattern of immunoreactivity has been reproduced a total of 3 times.)

The rise in plasma levels of β-EI was associated with a significant (~ 20%, $P < 0.01$ and ~25%, $P < 0.01$, respectively) decrease in the β-EI content of both the anterior and neurointermediate lobes of the pituitary (Fig. 2c, d). Figs. 2a and b, respectively, indicate the accompanying foot-shock-effected diminution in hypothalamic (~20%, $P < 0.01$) and PVA (~40%, $P < 0.01$) concentrations of β-EI.

In contrast, striatal, hypothalamic and medulla/pons levels of M-EI were unaffected by stress and a non-significant tendency for a decrease in levels seen in only the neurointermediate lobe (NIL) of the pituitary (Table I).

Rats rendered highly tolerant to morphine (ED_{50} about 300 mg/kg, cf. 7 mg/kg in placebos to s.c. applied morphine, as quantified by means of the vocalization analgesia test) possessed basal plasma levels of β-EI significantly ($P < 0.002$) less than those of placebo animals (Fig. 3). β-EI was, in fact, completely undetectable in morphine-dependent animals. (The relatively higher values of basal and foot-shocked levels in these placebo as compared to naive rats may reflect the chronic stress of the implantation procedure or possibly, age-dependent differences.) However, foot-shock elicited a substantial rise in the plasma content of β-EI in morphinized animals to a level not significantly differing from that determined in placebo rats and with the former group displaying, when considered in comparison to basal values, a relatively greater increase in levels post-stress (Fig. 3). This long-term morphine administration effected a 45% depression in the β-EI content of the NIL (placebo, 353 ± 27 ng/g tissue cf. morphine, 205 ± 12 ng/g, $P < 0.001$, n = 13 + 13), but no corresponding change in its anterior counterpart. These data are in agreement with those derived from a previous study employing a similar programme of treatment[40].

TABLE I

The influence of 5 minutes foot-shock upon the levels of Met-enkephalin immunoreactivity in particular structures of brain and pituitary

Means ± S.E.M. The data for neurointermediate lobe and hypothalamus represent the mean of 2, and for striatum and medulla, of 1, experiment. Total n: 14, 14, 6 and 8, respectively. No significant basal vs stressed differences.

	Neurointer-mediate lobe (nmol/g)	*Hypothalamus (pmol/g)*	*Striatum (pmol/g)*	*Medulla/pons (pmol/g)*
Basal, non-stressed	11.11 ± 1.10	709.31 ± 32.26	797.01 ± 64.36	278.60 ± 25.81
10 minutes post-stress	9.12 ± 0.93	714.42 ± 49.68	879.60 ± 64.21	274.09 ± 18.63

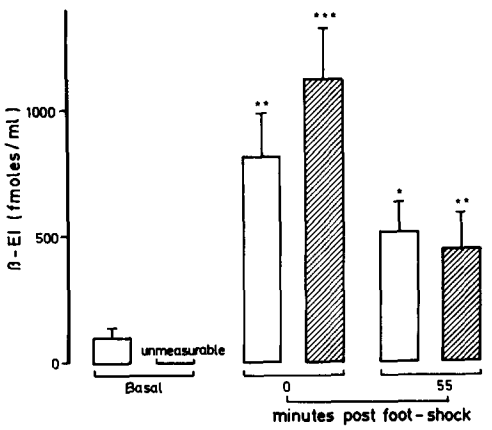

Fig. 3. Basal and stressed levels of β-endorphin immunoreactivity in the plasma of rats chronically treated with morphine ▨ or placebo □ pellets for 35 days. Bars indicate S.E.M. n = 5 per column. The significance of the difference between the placebo-morphine basal levels is $P \leq 0.02$. Asterisks indicate significance of basal vs stressed differences in placebo and morphine animals, respectively: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

The influence of naloxone upon basal nociceptive threshold, stress-induced analgesia and hyperthermia

Five minutes foot-shock evoked a large ($P < 0.001$) elevation in the tail-flick latency of naive rats, naloxone proving ineffective in modifying the pattern of analgesia, when injected immediately before stress at a dose of 1 or 10 mg/kg (not shown). However, when applied 10 min prior to stress, naloxone at 10 mg/kg exerted a small, but significant, degree of antagonism (Fig. 4a). This suggests that timing of administration is of considerable importance (see ref. 33), and for unclear reasons since receptor occupation and, for example, precipitation of withdrawal, is almost instantaneous (approx. a few minutes). In analogy, naloxone only attenuates the analgesia produced by microinjection of glutamate into the PVA of rats when administered 30, but not 5 min prior to this excitant[51].

The absence of any naloxone-elicited hyperalgesia upon re-estimation of tail-flick latencies 10 min after naloxone application can also be seen from Fig. 4a. The non-appearance of any increase in latency in saline rats post-injected should be noted. Thus, in order to circumvent the problem of the effect of the stress of handling and injection, all rats were adapted (three times) to the tail-flick/injection procedure prior to testing.

Fig. 4b illustrates the dose-dependent (1 and 10 mg/kg) capacity of naloxone, injected 5 min pre-stress, to moderate the elevation in Tc evoked by stress. These data are consistent with those of Bläsig et al.[7], who demonstrated stereospecific blockade by naloxone of the hyperthermic response to emotional (handling) stress.

The present data thus demonstrate the greater susceptibility to antagonism by naloxone of the hyperthermia, in comparison to the analgesia, produced by stress. However, the inability of 10 mg/kg naloxone (2 mg/kg was similarly ineffective) in altering the fall in Tc when applied 15 min post-stress at the peak of the rise in Tc, should also be noted (not shown).

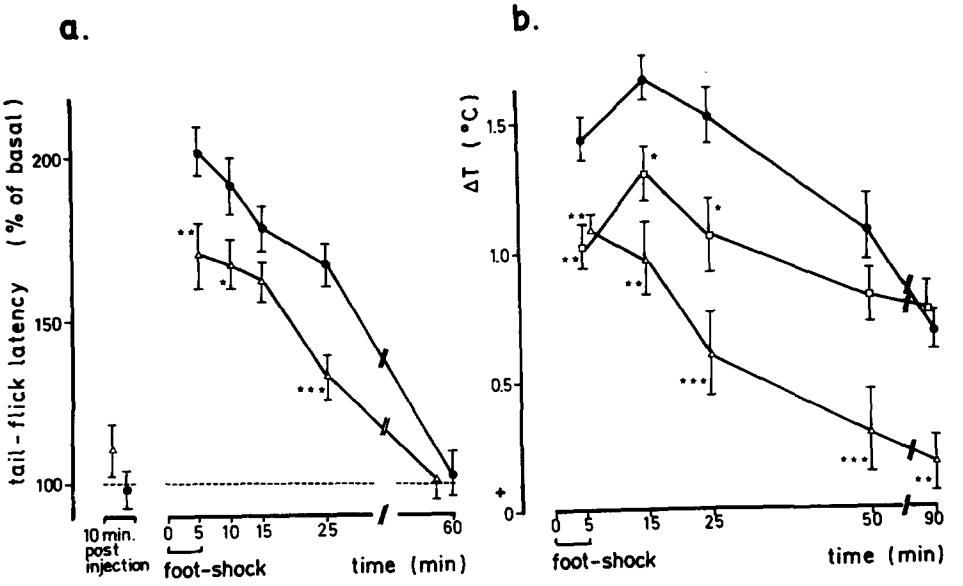

Fig. 4. a: the influence of 10 mg/kg naloxone upon pre- and post-stressed tail-flick latencies. Basal latencies were read, saline (●, n = 8) or naloxone (△, n = 8) administered, latencies re-evaluated 10 min post-injection and the animals immediately subjected to 5 min foot-shock. The increase in latency is expressed as a percentage of pre-injection basal values. Means ± S.E.M. Significance of saline–naloxone differences are indicated. Basal latencies (not significantly different) were: saline 3.60 ± 0.05 and naloxone 3.69 ± 0.08 sec, respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. b: the influence of 1 or 10 mg/kg naloxone applied 5 min prior to foot-shock upon the changes in core temperature (ΔTc) thus evoked. Saline (●, n = 10), 1 mg/kg naloxone (□, n = 8) and 10 mg/kg naloxone (△, n = 7). Means ± S.E.M. Basal core temperatures (no significant differences): 37.75 ± 0.09, 37.79 ± 0.10 and 37.86 ± 0.11 °C, respectively). Significance of saline–naloxone differences indicated: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

The influence of long-term morphine treatment upon stress-induced analgesia and hyperthermia

Although the basal nociceptive threshold of naive (matched weight), morphine- and placebo-treated rats did not differ significantly (as evaluated by the vocalization, in addition to the tail-flick tests), morphinized animals displayed a SIA of unusual persistence and enhanced intensity (Fig. 5a), with tail-flick values lying significantly ($P < 0.001$) above basal values for 90 min post-stress, and in excess of those of naive (not shown) and placebo animals at the 25 ($P < 0.01$), 60 ($P < 0.001$) and 90 ($P < 0.001$) min measurement time-points. Naive and placebo rats displayed comparable (not significantly different) patterns of SIA.

A significant smaller ($P < 0.01$) increase in Tc was seen after foot-shock in morphine- as compared to placebo-implanted rats, although the basal Tc values of morphine- and placebo-treated groups did not differ significantly. These data are not corroborative of those of Bläsig et al.[6], who reported the non-cross-tolerance of emotional hyperthermia to morphine. This discrepancy may reflect the use of a different stress model and/or the longer duration of implantation presently used (~ 30 cf. 10 days). This results in a more pronounced degree of tolerance (ED_{50} ~ 300 cf.

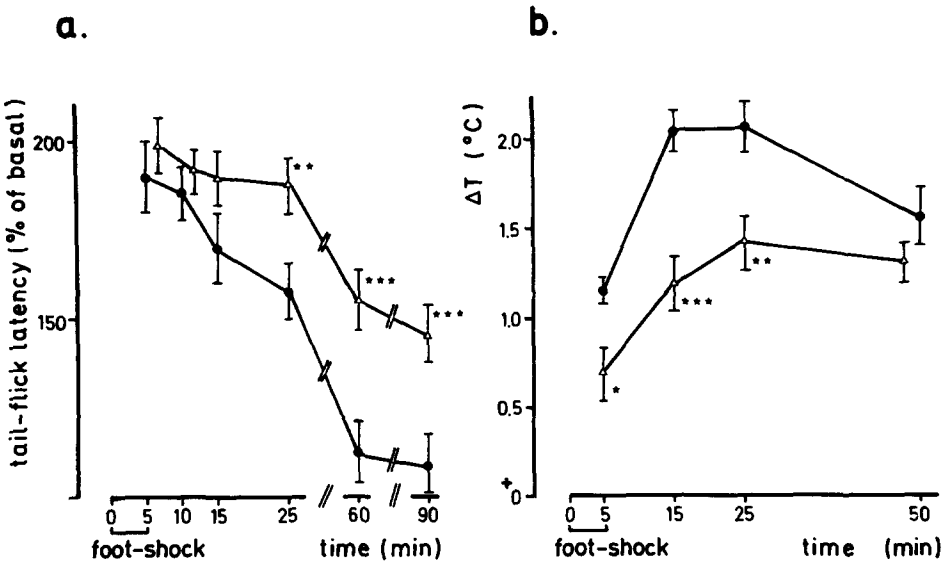

Fig. 5. a: the influence of 5 min foot-shock upon the tail-flick latency of placebo- (●, n = 6) and morphine-implanted (△, n = 7) (day 32 of implantation) animals. Means ± S.E.M. Basal latencies: (no significant differences) 3.61 ± 0.16 and 3.72 ± 0.09 sec, respectively. All morphine points significantly elevated above basal values. Significance of morphine-placebo differences indicated: ** $P < 0.01$, *** $P < 0.001$. b: the influence of 5 min foot-shock upon the core temperature (expressed as change (ΔTc) from basal) of placebo- (●, n = 7) and morphine-implanted (△, n = 10) rats on day 29 of implantation. Means ± S.E.M. Basal core temperatures (not significantly different) were 37.48 ± 0.14 and 37.30 ± 0.09 °C, respectively). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

~80 mg/kg morphine) changes in β-endorphin levels in the NIL and, possibly, the behaviour of endorphinergic and other systems responsive to stress, not produced by short-term administration.

DISCUSSION

The influence of foot-shock stress upon brain, pituitary and plasma levels of β-EI and M-EI

An increase in plasma levels of ACTH has been long established as a distinctive response to stress and a parallel rise in plasma levels of β-EI is now recognizable as equally characteristic[18,44]. Thus, Fig. 1a illustrates the anticipated large elevation in levels of plasma β-EI produced by exposure to 5 min foot-shock. Further, both in vitro[41] and in vivo[18] evidence has accumulated for the concomitant adenohypophyseal secretion of ACTH and β-EI, and the 20% fall in adenohypophyseal content of β-EI post-stress (Fig. 2c) is, therefore, indicative of a contribution of this lobe to the increase in plasma levels of β-EI detected.

Whereas the anterior lobe (AL) of the pituitary contains[24,41] and, in vitro, releases[41] approximately equal proportion of β-LPH and β-EP, the NIL, in contrast, contains and in vitro secretes predominantly (~95%) β-EP[24,41]. (It should be mentioned, however, that although the AL form of β-EP is apparently authentic

(Smyth, personal communication, Gordon Conference), in the NIL, β-EI consists primarily (80%) of the C'-fragment (β-LPH_{61-87}) and acetylated C'-fragment (both of which are opiate-inactive), indistinguishable from β-EP (β-LPH_{61-91}) by use of our antiserum[55].) The presence of β-LPH in plasma (Fig. 1b) constitutes unequivocal evidence of a mobilization of AL pools of β-EI into the circulation in response to stress. In previous studies, it was reported that β-EI in the plasma of stressed rats was composed entirely of β-EP[18,44], a finding which the present authors have also made when the interval between sacrifice of the rat and application of plasma to the column was not reduced to an absolute minimum (~10–15 min).

Notwithstanding the possibility of post-extraction (or in vivo) conversion of plasma β-LPH to β-EP, it is of interest in this connection that, whilst β-EP comprised the majority (~70%) of plasma β-EI (Fig. 1b), a 25% fall in the β-EI concentration of the NIL was detected post-stress (Fig. 2d). These observations suggest that the NIL may contribute to the rise in circulating levels of β-EI produced by stress. Further, a body of evidence has accumulated indicating that the NIL may discharge α-MSH, localized within the same cells as β-EP[39], into plasma upon challenge by a neurogenic stress such as foot-shock[36,48]. Although the report that 30 min foot-shock evokes a significant diminution in the levels of (uncharacterized) opiate activity in the AL, but not the NIL[2], is at variance with this hypothesis, Rossier et al., observed a 20% tendency for a decrease in the β-EI content of the NIL upon exposure of rats to 60 min foot-shock[43]. Further, the longer time-scales involved in the studies by these authors (in comparison to the present paper) may allow replacement synthesis of the pool of β-EP released.

The mobilization of β-EI into plasma was parallelled by a significant decrease in the β-EI content of both the hypothalamus, the perikaryonergic origin, and the PVA, a body of tissue which represents the primary projection target of the central network of β-endorphinergic neurones (Fig. 2a, b). These changes presumably reflect activation of this central pathway and can be considered as indicative of the release of β-EI. Whilst the releasability of the hypothalamic pool of β-EI has been established in vitro[38], Rossier et al.[44] have similarly interpreted the fall in hypothalamic levels of β-EI instigated by 30 min foot-shock. The present demonstration of stress-induced activation of the PVA β-endorphinergic input is particularly pertinent to the phenomenon of SIA. Thus, the PVA is peculiarly responsive to the naloxone-reversible and cross-tolerant[31] analgesic effects of both opiate microinjection and electrical stimulation[54].

Met-enkephalin may also be involved in central analgesic (see ref. 17) and/or thermoregulatory[6] processes, but the M-EI content of the NIL, striatum and medulla/pons remained stable under stress (Table I). Further, in common with Fratta et al.[14], but in distinction to Rossier et al.[45], who reported a 20% stress-evoked fall in the levels of Leu-enkephalin immunoreactivity (L-EI), no significant foot-shock-elicited depletion of hypothalamic M-EI was detectable (Table I). The discrepancy between these studies may be related to the fact that Rossier et al.[45] determined levels of L-EI rather than M-EI. Thus, although in vitro (in striatum) a parallel stimulated outflow of M-EI and L-EI is seen[37], the presence of distinct Met- and Leu-enkephalin-

ergic neurones in the hypothalamus, as apparently exists in other tissues[28], might allow for their differential activation.

As shown in Fig. 3, basal plasma levels of β-EI in morphinized rats were significantly lower than those of placebo animals, consonant with the depressed levels of plasma and urinary corticosteroids observed in both chronically morphinized rats[10] and heroin addicts[13]. (Interestingly, it has recently been demonstrated that human heroin addicts similarly possess decreased plasma levels of β-EI compared to 'normal' subjects[21].) However, as previously determined for ACTH in morphine-dependent rats[26], stress evoked a considerable increase in plasma levels of β-EI, not significantly different from that revealed by placebo animals (Fig. 3). Thus, the selective decrease in NIL and basal plasma levels of β-EI precipitated by long-term morphine administration is not accompanied by any suppression of the capacity of the pituitary to discharge β-EI upon challenge by an acute stress, a situation which may, by analogy, also be apparent in humans addicted to opiates.

The influence of naloxone and chronic morphine treatment upon basal nociceptive threshold and stress-induced analgesia

The inability of naloxone to modify basal nociceptive threshold and its marginal (but significant) attenuation of SIA (see Fig. 4a) is consistent with the balance of evidence documented in the literature which reveals a recurrent controversy as to the potency of naloxone in influencing either of these measures.

Since SIA in rats is refractory to the dose of 1 mg/kg naloxone[2,17,33,42], sufficient to abolish the analgesic and other behavioural effects of, for example, i.c.v. injected opiates and endorphins, and since 10 mg/kg was only slightly efficacious in attenuating this SIA, opiates appear to subserve no dominant role in the generation of SIA (Fig. 4a). (This pattern of results has recently been replicated by the present authors by use of the hot-plate test, see ref. 33.) The observation that sub-total lesions of the spinal cord, which reduce both morphine and stimulation-produced analgesia, do not alter the patterns of SIA in rats is supportive of this assertion[4,19]. Further, treatment of rats with either dexamethasone or metyrapone (which respectively abolish and enhance the release of corticotrophic pools of β-EI)[15] does not modify SIA[35,42] while radiofrequency destruction of central β-endorphin perikarya only slightly attenuates SIA[34,42]. These observations specifically suggest that β-endorphin is not the major contributor to the development of this analgesia.

The importance of interspecific differences in the function of opiate systems should, however, be emphasized. Thus mice almost invariably exhibit a naloxone-inducible hyperalgesia and a SIA both substantially attenuated by 1 mg/kg naloxone and cross-tolerant to morphine. In contrast, naloxone hyperalgesia can (relatively) only seldom be elicited in rats, in which at most a partial antagonism of SIA is exerted by even 10 mg/kg naloxone, and a reduction of naloxone-sensitive SIA is not detectable in morphine-dependent animals[2,11,17,33,43].

Further, the expression SIA demands some qualification, and whilst the tail-flick test does apparently reflect the intensity of pain-perception[19], endorphins may modulate the response to and the ability to become tolerant to, compared with the ability to

perceive a noxious stimulus[2]. Moreover, in contrast to short duration stresses, longer-term treatments[32] may be more adept at eliciting a naloxone-sensitive 'analgesia'. (The influence of variation in these and other factors upon SIA is considered in detail in ref. 33.)

Nevertheless, endorphins appear to fulfill no major role in the regulation of pain-perception under acute stress in rats, and that any such action if not indispensable is indicated in the absence of cross-tolerance of SIA to morphine[9] (Fig. 5a). (It is of interest to note that naloxone-antagonizable acupuncture analgesia has similarly been found to be non-cross-tolerant to morphine[12].) It is improbable that an analgesic endorphinergic mechanism is operative in morphinized rats, in view of the cross-tolerance exhibited by opioid peptides[16,50] and naloxone-blockable analgesic stimulation of the PVA[31] to morphine. Thus, the amplified SIA seen may represent a compensatory hyperactivation of (a) non-endorphinergic system(s), the existence of which is reflected in the large non-naloxone-susceptible component of SIA. It is of interest that evidence has been provided for changes in, for example, pituitary secretion[53], catecholamine metabolism[5] and sensitivity to transmitters[47] in chronically morphinized animals.

The influence of naloxone and long-term morphine treatment upon stress-induced hyperthermia

In view of the biphasic influence of morphine upon Tc, with hyperthermia at low and hypothermia at high doses, respectively[6,20,22,25,29,46], an endorphin mediation of either heat production or dissipation might be anticipated. The present stress model instigates an activation of endorphinergic systems (Figs. 1 and 2) which parallels the dose-dependent naloxone attenuation of SIH (Fig. 5b), and a causal relationship between these stress responses appears probable. Although the particular pool(s) responsible remain(s), as yet, unidentified, the inability of either selective ablation of the NIL or dexamethasone administration (which blocks the stress-evoked release of prolactin in addition to ACTH and β-EI[15]) to attenuate SIH demonstrates that pituitary pools of these peptides do not mediate the hyperthermic response to stress[33]. Since neither 2 nor 10 mg/kg naloxone applied 15 min post-stress affected the subsequent fall in Tc (not shown), no participation of endorphins in the restoration of basal Tc post-stress is indicated. However, the possibility that a hypothermic action of endorphins is masked in these conditions cannot be discounted. Further, Holaday et al. have provided evidence that endorphins may act as hypothermic determinants at least under heat stress[23].

The possible importance of the factor of enhanced muscular activity in the effects of opiates upon Tc, and in the development of SIH, should be considered. However, morphine has been shown to both cause vasoconstriction in rats [46] and hyperthermia in paralyzed cats[52]. Further, naloxone (10 mg/kg) does not greatly alter motor behaviour either during or subsequent to foot-shock (Millan, unpublished) whilst a hyperthermic action of morphine has been shown not to be dependent upon an increase in motor activity[30]. These points suggest that, rather than a generalized excitation, there may be a direct influence of endorphins upon a Tc-regulatory

mechanism in the generation of SIH, and possibly in tonic control of Tc[49]. Indeed, injection of β-EP directly into the hypothalamus, wherein the 'central thermostat' is located, does produce hyperthermia[29]. However, naloxone modifies neither the thermoregulatory response to cold nor pyrogen-induced hyperthermia[6].

The apparent cross-tolerance between endorphins and morphine[16,50], most probably explains the reduction in SIH seen in chronically implanted rats. However, an enhanced hypothermic effect of i.c.v. applied acetylcholine has been detected in morphine-dependent rats[27], the possibility of a supersensitivity or some other change in a non-endorphinergic thermoregulatory mechanism recruited by stress should not, therefore, be discounted.

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