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Adrenal Medullary Enkephalin-Like Peptides May Mediate Opioid Stress Analgesia

Abstract. *Different patterns of foot shock activate opioid and nonopioid mechanisms of stress analgesia in the rat. Opioid, but not nonopioid, stress analgesia is reduced by adrenal demedullation and denervation and is potentiated by reserpine, a drug known to increase concentrations of adrenal medullary enkephalin-like peptides. It is suggested that adrenal enkephalins mediate opioid stress analgesia.*

Enkephalins and larger enkephalin-like peptides are synthesized and stored, along with catecholamines, in chromaffin granules of the adrenal medulla (*1, 2*). Although the functional role of these opioids is not known, the fact that they are released by sympathetic activation or trauma (*2, 3*) suggests that they serve in the adaptive response to stress. The perception of pain is normally adaptive, impelling and guiding the organism into appropriate defensive behavior. However, pain suppression might prove more adaptive under conditions of emergency, when attention to noxious stimuli could disrupt effective coping (*4*). In fact, certain stressors are now known to cause potent analgesia in rodents (*5*). We previously found that varying only the temporal pattern of foot shock stress can determine whether an opioid or nonopioid form of analgesia occurs (*6*). We now provide evidence that adrenal medullary enkephalin-like peptides play a significant role in mediating opioid stress analgesia.

Seventy-two male Sprague-Dawley rats (350 to 400 g) were maintained on a 12-hour light cycle. The rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally) and subjected to adrenalectomy (N = 16), adrenal demedullation (N = 24), denervation of the adrenal medulla by celiac ganglionectomy (N = 8), or sham surgery (N = 24). For the remainder of the experiment, the adrenalectomized rats were given 0.9 percent saline to drink. Two weeks after surgery, the rats were exposed to prolonged, intermittent or brief, continuous foot shock during the dark phase of the light cycle. These stress regimens cause opioid and nonopioid analgesia, respectively (*6*). Prolonged foot shock was 20 minutes of intermittently applied 60-Hz sine-wave pulses (one 2.5-mA, 1-second pulse every 5 seconds); brief foot shock was 3 minutes of continuously applied 60-Hz sine waves (2.5 mA). Pain responsiveness was measured by the tail-flick method (*7*), with exposure to the radiant heat limited to 7 seconds. Baseline latencies were determined immediately before prolonged foot shock and 17 minutes before brief foot shock in order to keep the interval between baseline testing and the termination of stress constant for all subjects. Immediately after foot shock stress, pain responsiveness testing was resumed. Tail-flick trials were conducted for 9 minutes at 1-minute intervals and then at 2-minute intervals for three more trials. Each rat ultimately received both stress procedures, administered in a counterbalanced fashion and separated by 1 week.

One week after the second exposure to stress some rats from each group were tested for analgesic responsiveness to morphine (2.5 mg/kg, subcutaneously). Pain responsiveness (calculated as the mean latency measured in three tail-flick trials separated by 1 minute) was determined once before morphine injection and again 30, 60, 120, and 180 minutes after drug administration. The limit of exposure to radiant heat was extended to 16 seconds in these trials. Finally, the effect of adrenal demedullation or denervation on adrenocortical function was assessed by measuring serum corticosterone. Additional groups of 18 rats received adrenal demedullation, adrenal denervation, or sham surgery. Two weeks later, a time corresponding to that at which the behaviorally tested rats received their first analgesia test, animals from each group were decapitated and trunk blood was collected from nonstressed rats (N = 6) or immediately after prolonged (N = 6) or brief (N = 6) foot shock. Serum samples were stored and corticosterone levels were determined later by radioimmunoassay (*8*). All data were compared by analysis of variance, and Newman-Keuls tests were used for specific comparisons between groups (*9*).

Both prolonged and brief stress elicited potent analgesia in sham-operated rats (Fig. 1). The groups differed significantly in their response to prolonged stress ($P < .01$) but not to brief stress. Specific comparisons indicate that adrenalectomized, adrenal-demedullated, and adrenal-denervated groups all showed less analgesia after prolonged stress than the control group ($P < .01$ in

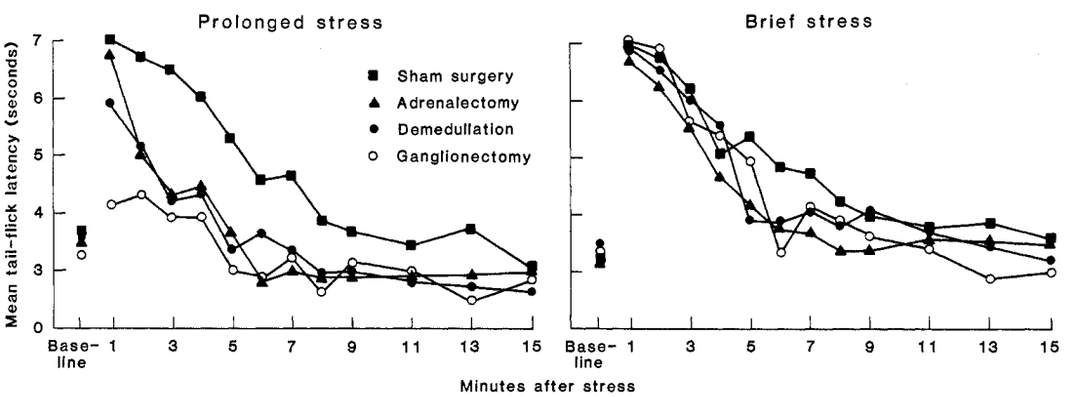

Fig. 1. Effects of adrenalectomy, adrenal demedullation, denervation of the adrenal medulla by celiac ganglionectomy, and sham surgery on the analgesic response to prolonged (opioid) and brief (nonopioid) foot shock. All three experimental procedures significantly attenuated opioid stress analgesia ($P < .01$) but not nonopioid stress analgesia.

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Table 1. Analgesic response to morphine (2.5 mg/kg, subcutaneously). Values are mean tail-flick latencies (seconds) ± standard errors.

Group	Baseline	Minutes after morphine injection			
		30	60	120	180
Adrenalectomy ($N = 8$)	3.7 ± 0.2	15.0 ± 0.6*	15.0 ± 0.4*	15.0 ± 0.4*	10.2 ± 0.8*
Adrenal demedullation ($N = 8$)	3.6 ± 0.2	9.8 ± 0.8	8.7 ± 0.7	5.8 ± 0.7	4.0 ± 0.4
Adrenal denervation ($N = 4$)	3.4 ± 0.3	9.5 ± 0.7	8.0 ± 0.8	5.3 ± 0.8	4.1 ± 0.6
Sham surgery ($N = 8$)	3.9 ± 0.2	9.8 ± 0.6	8.0 ± 0.8	5.7 ± 0.5	4.7 ± 0.5

*Significantly different from corresponding control value ($P < .01$).

each case) but did not differ from each other.

All groups showed clear morphine analgesia. The adrenalectomized rats manifested significantly more analgesia than the controls ($P < .01$); the other groups did not differ from one another in analgesia (Table 1). Radioimmunoassay revealed that basal corticosterone concentrations were unaffected by adrenal demedullation or denervation. Prolonged, but not brief, foot shock caused a significant increase in corticosterone levels in all groups [$P < .05$ for all groups compared to appropriate basal values (*10*)]. Although the corticosterone response of adrenal-denervated rats to prolonged stress was normal, the analgesic response was virtually absent (Fig. 1). This dissociation argues against a critical role for corticosterone in the mediation of opioid stress analgesia.

These findings show that the adrenal gland has a major role in the mediation of only the opioid form of stress analgesia. More particularly, it appears that the adrenal medulla is responsible for these effects in that denervation or removal of the medulla is as effective in blocking opioid stress analgesia as complete adrenalectomy. The effects of medullary denervation or excision cannot be attributed to compromised adrenocortical function, since adrenal denervation did not affect the concentration of corticosterone in serum before or after stress and since rats with demedullated or denervated adrenals failed to display the enhanced morphine analgesia characteristic of adrenalectomized animals (*11*). The findings by others that adrenalectomy attenuates a naloxone-sensitive form of stress analgesia (*12*) but does not affect a naloxone-insensitive form (*13*) are consistent with the present findings, although those studies suggested adrenal cortical, not medullary, involvement.

Having implicated the adrenal medulla in a form of stress analgesia that appears dependent on opioid peptides, we next sought to determine whether adrenal medullary opioids or catecholamines [with which opioids are released (*2*, *14*)] are critically involved. Six groups of eight rats were injected intraperitoneally with reserpine (Serpasil; 2 mg/kg) or saline on two successive days. Twenty-four hours after the last injection, the rats were tested for analgesic responsiveness to prolonged or brief stress, as before. A similar injection protocol was reported to deplete adrenal catecholamines while increasing the concentration of adrenal enkephalin-like peptides in several species (*15*). We recently found that this regimen of reserpine administration causes a nearly twofold increase in opiate-like materials in the rat adrenal medulla (*16*). Stimulated release of these peptides is still possible after reserpine, and the amount released is proportional to their new, elevated concentration (*17*).

The reserpine-treated animals displayed significantly greater analgesia after prolonged stress than the controls ($P < .01$) (Fig. 2). This enhanced analgesic response still appears to be mediated by opioids, since one group of reserpine-treated rats given the opiate antagonist naltrexone (3 mg/kg, subcutaneously) before prolonged foot shock showed significantly less analgesia than saline-treated controls ($P < .01$) (Fig. 2). In agreement with our previous findings (*18*), brief stress analgesia was refractory to blockade by an opiate antagonist (Fig. 2). This nonopioid analgesia was reduced by reserpine ($P < .05$) (Fig. 2), suggesting mediation by monoamines. The critical monoamine appears to be histamine (*19*).

The fact that denervation and excision of the adrenal medulla reduced opioid stress analgesia to the same extent as total adrenalectomy suggests that all three surgical effects are attributable to adrenal medullary damage. Postsurgical serum corticosterone levels indicate that medullary denervation does not disrupt adrenal cortical function. Opioid but not nonopioid stress analgesia is potentiated by reserpine given in accordance with a regimen that elevates adrenal enkephalin-like peptides, and even this potentiated analgesia is blocked by an opiate antagonist. Moreover, prolonged but not brief stress depletes opiate-like material in the adrenal medulla (*16*), and hexamethonium, a peripherally acting ganglionic blocking agent that reduces adrenal enkephalin secretion in vitro (*15*), attenuates opioid stress analgesia (*20*). These converging lines of evidence suggest that

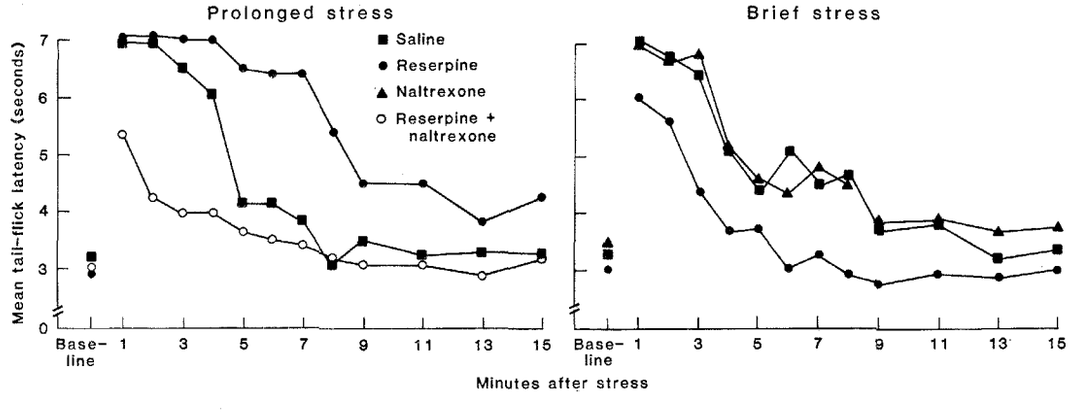

Fig. 2. Effects of reserpine and naltrexone on the analgesic response to prolonged (opioid) and brief (nonopioid) foot shock. Reserpine treatment significantly enhanced the analgesic response to prolonged stress ($P < .01$). Even in the presence of reserpine, naltrexone antagonized this analgesia ($P < .01$). Brief stress analgesia was significantly attenuated by reserpine ($P < .05$) but was not affected by naltrexone.

enkephalin-like peptides of the adrenal medulla are critically involved in mediating opioid stress analgesia in the rat (*21*).

Enkephalin and another small opioid released by the adrenal medulla cause analgesia when administered intracerebrally, but fail to do so after systemic injection, presumably because of their rapid enzymatic degradation (*22*). Consequently, these substances appear incapable of mediating stress analgesia. Other, longer chain enkephalins are also released by the adrenal medulla, however, and some of these bind to opiate receptors (*23*). Still other adrenal peptides lack opiate activity until enzymatically cleaved into smaller fragments (*24*). It may be that such longer chain opioids are protected from degradation and hence reach sites of pain inhibition in the central nervous system before enzymatic cleavage occurs.

The results of this study present an interesting paradox: adrenalectomy blocks an opioid form of stress analgesia but potentiates the analgesic response to morphine. Unlike stress analgesia, however, morphine analgesia is unaffected by adrenal demedullation and denervation; it seems that the adrenal cortex, not medulla, is involved. Other findings support this view. For example, hypophysectomy, which causes adrenal cortical atrophy, potentiates morphine analgesia as does adrenalectomy (*25*), and normal morphine responsiveness is restored by hormonal replacement (*26*). Also, the affinity of opiate receptors to etorphine or morphine is increased by both adrenalectomy and hypophysectomy (*27*). It seems, therefore, that adrenalectomy potentiates opiate analgesia by increasing the affinity of receptors for opiate drugs in response to the loss of adrenocortical hormones, but decreases opioid stress analgesia by eliminating an important source of opioid peptides, the adrenal medulla.

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