Multiple Neurochemical and Hormonal Mechanisms of Stress-Induced Analgesia[a]

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The principal theme of our work on the phenomenon of stress-induced analgesia has been that multiple neurochemically and neurohormonally discrete pain-inhibitory systems exist and that these systems can be selectively activated by a single stressor, inescapable footshock. Some of these stress-activated endogenous mechanisms of analgesia involve opioid peptides, others do not. Similarly, some forms of stress analgesia rely principally on central nervous system substrates, but others are dependent upon hormonal factors, possibly opioid peptides, as well. This chapter describes our initial investigations identifying these various forms of stress analgesia, highlights some of the evidence indicating the independence of these multiple forms, and attempts to integrate some of our findings with those reported by others.

EVIDENCE FOR MULTIPLE ENDOGENOUS ANALGESIA SYSTEMS

That portions of the central nervous system have, as their normal function, the inhibition of pain was clearly indicated by the observations of Reynolds[1] and Mayer *et al.*[2] that electrical stimulation of the periaqueductal gray region of the medial brainstem causes profound analgesia. Subsequently, the report of Akil *et al.*[3] that this analgesia is blocked by administration of an opiate antagonist drug provided compelling evidence for the existence of an endogenous, opioid-mediated analgesia system. More recently, Cannon *et al.*[4] have demonstrated the existence of neuroanatomically distinct, nonopioid pain-inhibitory systems. They confirmed the finding of Akil *et al.*[3] that stimulation of particular brain loci can cause opioid-mediated analgesia and also showed that stimulation of anatomically adjacent sites can elicit an equally potent, non-opioid-mediated analgesia.

In 1976, Hayes *et al.*[5] and Akil *et al.*[6] found that exposure to environmental stressors could activate endogenous mechanisms of antinociception. Both groups entertained the hypothesis that stress-induced analgesia may be mediated by the recently discovered opioid peptides. Their conclusions, however, were quite

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different. Akil *et al.*[6] found that the analgesia induced by exposure to 30–60 minutes of intermittent footshock was prevented by an opiate antagonist drug, suggesting the involvement of opioids. Hayes *et al.*[5,7] using a variety of stressors, including footshock of brief duration, observed no effect of opiate antagonist drugs and concluded that stress analgesia was mediated by non-narcotic systems. Subsequently, several investigators confirmed the finding that exposure to stress can elicit analgesia in laboratory animals,[8-11] although taken together, their results were inconclusive regarding the activation of opioid systems by environmental stimuli. The use of qualitatively different stressors, or quantitatively different applications of the same stressor, in these studies, however, made a general reconciliation of the data difficult.

SELECTIVE ACTIVATION OF OPIOID AND NON-OPIOID PAIN-INHIBITORY SYSTEMS BY FOOTSHOCK STRESS

When we began our investigations of stress analgesia in 1979, our goals were to clarify what role, if any, was played by the opioid peptides in this phenomenon, and to identify and characterize non-opioid-mediated forms of stress analgesia. To accomplish this, we chose to employ a single stressor, inescapable footshock. Using modifications of the intermittent footshock procedure described by Akil *et al.*[6] and the continuous footshock paradigm of Hayes *et al.*,[7] we demonstrated that a single stressor could elicit either an opioid-mediated or a non-opioid-mediated analgesic response depending upon the parameters of its application. The analgesia induced by intermittent footshock was antagonized by pretreatment with low doses of naloxone,[12,13] suggesting opioid involvement. The analgesia following exposure to continuous footshock was equipotent, but refractory to even high doses of naloxone.[12] To explore further the opioid and non-opioid characteristics of these two forms of stress analgesia, experiments were conducted to test for (1) the development of tolerance upon repeated stress exposure; (2) cross-tolerance between stress analgesia and morphine analgesia; and (3) cross-tolerance between these two forms of stress analgesia. Consistent with the hypothesis that intermittent footshock-induced analgesia is mediated by opioid systems, we found that tolerance develops to this form of footshock after 14 daily exposures,[14,15] and that this form of stress analgesia is nearly absent in animals rendered tolerant to morphine.[14] By contrast, there was no evidence of the development of tolerance to the continuous footshock stressor, nor was this form of stress analgesia affected in morphine-tolerant rats.[14] Finally, further testifying to the independence of the pain-inhibitory systems subserving these two forms of stress analgesia was the finding of no cross-tolerance between these stressors.[16] Thus, multiple endogenous pain-inhibitory systems exist and they can be selectively activated by different parameters of footshock administration.

Continuous Footshock-Induced Analgesia

In our early work, we reported that the analgesia following exposure to 3 minutes of continuous footshock (2.5 mA) was non-opioid in nature.[12] Subsequently, work of Terman *et al.*[17] has extended these observations and indicated that the analgesic response to continuous footshock is not unitary, rather its neurochemical basis is very dependent upon both the intensity and duration of

the footshock stimulus. If footshock duration is fixed (e.g., 3 minutes), low current intensities (1–2 mA) elicit an opioid analgesia, whereas exposure to higher intensities (2.5–3.5 mA) results in a non-opioid response. Similarly, if current intensity is held constant (e.g. 2.5 mA), sessions of brief duration (1–2 minutes) produce opioid analgesia, and those of longer duration (4–5 minutes) activate non-opioid mechanisms. The opioid and non-opioid nature of these two forms of stress analgesia was inferred using the same criteria as before (e.g. antagonism by naloxone, development of tolerance, and manifestation of cross-tolerance with morphine analgesia). Thus, once again, the opioid or non-opioid basis of stress analgesia is critically dependent upon the parameters of the stressor. In the case of continuous footshock, it is the interaction of intensity and duration that appears to define which pain-inhibitory systems are accessed.

Although neurochemically different, these two forms of stress analgesia depend on similar anatomical substrates. Both forms are reliant on brainstem and descending neural systems; they are blocked by spinal transection and are unaffected by decerebration.[18,19] Moreover, both forms of stress analgesia appear to be mediated principally by neural systems since they are affected neither by hypophysectomy nor adrenalectomy.[17,20,21] Interestingly, Terman *et al.*[17] have found that it is possible to elicit opioid and non-opioid stress analgesia in pentobarbital-anesthetized rats. That is, the analgesic response to 1 or 4 minutes of continuous footshock displayed by anesthetized rats is indistinguishable, in magnitude or duration, from the behavior emitted by awake animals. This observation attests to the importance of lower brain structures, those not affected by pentobarbital, in the organization of these forms of stress analgesia, and opens the possibility of studying these behaviors in anesthetized animals to technical and ethical advantage.

Intermittent Footshock-Induced Analgesia

As we[12] and Akil *et al.*[6] have shown, the analgesic response to intermittent footshock stress appears to be dependent upon opioid systems. This analgesia is similar to the opioid analgesia elicited by continuous footshock in that these two forms of stress analgesia manifest cross-tolerance with each other, but not with the non-opioid form.[18] This form of stress analgesia also shares a common neuroanatomy, the dorsolateral funiculus of the spinal cord, with both forms induced by continuous footshock.[18,22]

The neurochemical and hormonal mediation of this opioid stress analgesia, however, is distinct from the other forms. For example, acetylcholine has long been thought to be involved in central mechanisms of antinociception.[24] We have found that the opioid analgesia caused by intermittent footshock, but not the opioid or non-opioid analgesic responses to continuous footshock, is reduced by scopolamine, a muscarinic cholinergic antagonist drug.[18,24] Importantly, methylscopolamine, a muscarinic cholinergic antagonist with only peripheral activity, failed to affect the analgesic response to intermittent footshock. That acetylcholine serves to stimulate the release of opioid peptides involved in pain inhibition is suggested by the finding that oxotremorine, a potent muscarinic agonist, causes analgesia sensitive to opiate antagonist blockade.[23–25] We therefore conclude that a muscarinic cholinergic synapse exists in the central pathway mediating some, but not all, forms of opioid stress analgesia.

Role of Pituitary-Adrenal and Sympatho-Adrenal Hormones

Regarding the hormonal mediators of stress analgesia, several groups have identified a key role for pituitary factors. Hypophysectomy has been shown to markedly reduce many forms of stress analgesia,[26-29] particularly those forms sensitive to naloxone blockade. This surgical manipulation, however, has been found ineffective in attenuating some opioid[17,30] and most non-opioid[17,30,31] forms of stress analgesia. Consistent with such findings, we have reported that hypophysectomy reduces intermittent footshock-induced analgesia, but enhances non-opioid stress analgesia.[20]

In our original report[12] we found that administration of the synthetic glucocorticoid, dexamethasone, powerfully antagonized the analgesic response to intermittent footshock stress. At that time, the elimination of the analgesia was attributed to inhibition of the release of pituitary β-endorphin. Since this time, however, several findings have caused us to re-evaluate this hypothesis. First, since the magnitude of the reduction in opioid stress analgesia caused by hypophysectomy is modest compared to that due to dexamethasone treatment, it is possible that steroids exert their antagonistic effects at other, extra-pituitary, loci. Second, there is considerable evidence to suggest that corticosteroids can interact with opioid systems,[32] and we have shown that chronic treatment with dexamethasone sensitizes rats to the analgesic effects of morphine (unpublished observations). Finally, Chatterjee *et al.*[33] demonstrated that glucocorticoids can have opposite effects on opiate action. Depending upon the dose given and time of administration relative to morphine challenge, low doses can potentiate and high doses can antagonize morphine analgesia. It may be that the effect of hypophysectomy, in our experiments, is due to the reduction in stress-induced release of steroids, not opioids.

To test the hypothesis that adrenal steroids can interact with opioid pain-inhibitory systems, we assessed the effects of corticosterone administration on opioid analgesia induced by continuous footshock.[34] We chose this form of stress analgesia since it shares common mechanisms (i.e. is cross-tolerant with) that opioid form induced by intermittent footshock, but it is not dependent upon pituitary or adrenal factors.[17] Opioid stress analgesia was enhanced by pretreatment with low doses of corticosterone and antagonized by high doses. That these effects are mediated by corticosteroid action in the brain, not the pituitary, is indicated by the finding that hypophysectomy affected neither the analgesic response to continuous footshock nor the potentiating or antagonizing effects of corticosterone. Thus, it appears likely that adrenal steroid hormones serve either as critical mediators[29] or modulators[12,34] of opioid forms of stress analgesia.

Another peripheral source of opioid peptides is the adrenal medulla. The adrenal medulla contains enkephalin-like peptides that are stored and co-released with catecholamines.[35] Although the precise physiological function of these peptides remains to be determined, we have suggested that they are importantly involved in the analgesic response to certain forms of stress. Intermittent, but not continuous, footshock-induced stress analgesia is markedly reduced by adrenalectomy, adrenal demedullation, or denervation of the adrenal medulla via celiac ganglionectomy.[21] Because demedullation and ganglionectomy have as great an effect as removal of the entire adrenal gland, and because both basal and stressed adrenocortical functions were unimpaired in the adrenal denervated animals yet these rats failed to manifest opioid stress analgesia, we

concluded that this form of stress analgesia depends specifically upon adrenal medullary, not cortical, function. Moreover, enkephalin-like peptides but not catecholamines, appear to be involved in this response. A dose of reserpine, known to increase the adrenal content of enkephalins and their stimulation-induced release,[36] significantly augments opioid stress analgesia. This enhanced analgesia appears to reflect increased release of enkephalin-like peptides by stress rather than a nonspecific drug effect in that the analgesia is still virtually eliminated by an opiate antagonist drug.

Biochemical correlates of these behavioral observations have been measured in a collaborative study with Dr. O.H. Viveros.[37] The amount of opiate-like material in adrenal medulla was significantly reduced by intermittent, but not by continuous, footshock, suggesting that acute exposure to this stressor results in the release of enkephalin-like peptides. Medullary enkephalin content was dramatically increased in reserpine-treated rats. This new elevated content was also reduced by exposure to intermittent footshock. Finally, rats made tolerant to intermittent footshock analgesia no longer showed a depletion of adrenal enkephalin-like peptides after stress. These several converging lines of evidence strongly implicate adrenal enkephalin-like peptides in opioid stress analgesia.

Possible Involvement of the Nucleus Tractus Solitarius in Opioid Stress Analgesia

Although we have provided clear evidence for involvement of adrenal opioids in intermittent footshock-induced analgesia, several important questions, such as the locus of the opiate receptor mediating this analgesia, remain to be answered. It may be that enkephalins of adrenal origin are transported to the central nervous system and act upon opioid pain-inhibitory systems. While this hypothesis cannot be discounted, it is unlikely due to the relatively short half-life of these peptides in plasma. An alternate mechanism, based upon peripheral activity of enkephalins, has been suggested by the work of Maixner and Randich.[38,39] They have shown that analgesia elicited either by intermittent footshock or systemic administration of enkephalins is attenuated by unilateral vagotomy. These findings imply that enkephalin-like peptides, secreted by the adrenal medulla, cause peripheral effects, possibly alterations in hemodynamics, and that information regarding these perturbations is sent to the central nervous system via the vagus.

A recent finding in our laboratory may extend this neural circuitry to the brain. One of the principal central nervous system projections of the vagus is the nucleus tractus solitarius (NTS), a nucleus located in the lower brainstem well known to be involved in autonomic control.[40] Several lines of evidence suggest that the NTS may be an integral part of endogenous pain-inhibitory systems. Biochemically, this region is rich in opioid peptides and their receptors,[41,42] and anatomically it has extensive projections to, or receives afferents from, several brain loci thought to be important for pain inhibition.[43] We have recently found that electrical stimulation of the nucleus tractus solitarius causes opioid-mediated analgesia in rats.[44] Thus, since exposure to stress is accompanied by a host of autonomic sequelae, and because the NTS is involved in these responses and NTS stimulation causes analgesia, it is reasonable to hypothesize that an important linkage between stressful stimuli and endogenous analgesia systems occurs via the nucleus tractus solitarius.

FIGURE 1 provides a schematic diagram detailing several of the neural and

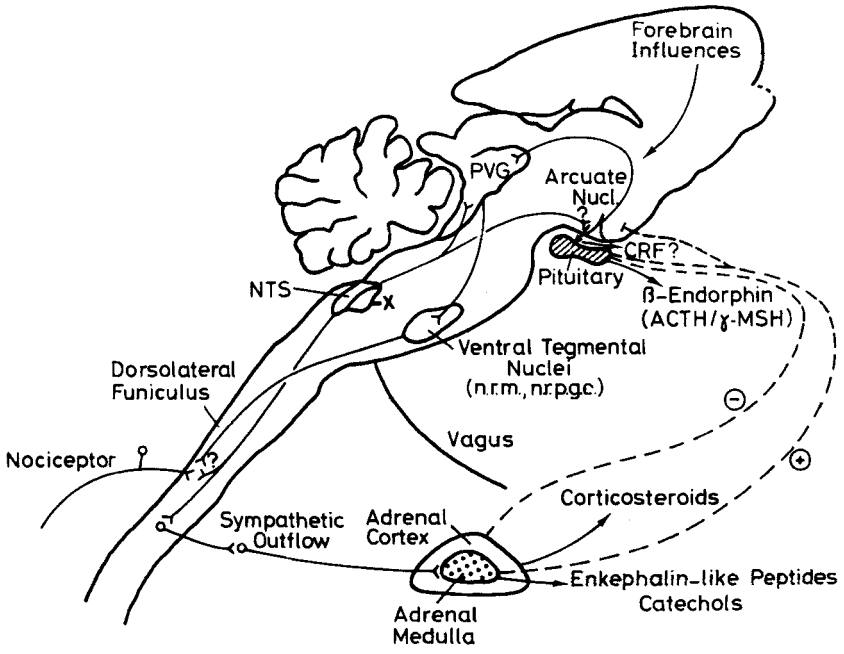

FIGURE 1. This schematic diagram illustrates several of the neural systems and endocrine factors thought to be involved in some forms of opioid-mediated stress analgesia. Abbreviations: ACTH, adrenocorticotropic hormone; CRF, corticotropin releasing factor; MSH, melanocyte stimulating hormone; NRM, nucleus raphe magnus; NRPGC, nucleus reticularis paragigantocellularis; NTS, nucleus tractus solitarius; PVG, periventricular/ periaqueducatal gray matter; X, nucleus of the vagus.

endocrine structures and factors thought to contribute to the various forms of footshock-induced analgesia.

Involvement of Central Nervous System Opioids

That adrenal opioids are critical to intermittent footshock-induced analgesia by no means precludes the involvement of central opioids as well. In fact, many investigators have shown alterations in brain opioid content following exposure to stress.[15,45-47,69] One experimental strategy that has been employed in an attempt to quantify stress-induced release of opioids in the brain involves either *in vivo* administration of radioactive opiate drugs or submission of brain homogenates of normal and stressed rats to *in vitro* opiate receptor binding procedures. The predicted outcome of such experiments is that opioids released by stress will occupy receptor sites in the brains of stressed animals and inhibit the binding of exogenously applied radioactive opiates. Using these procedures, several investigators have observed decreased radioactive ligand binding in stressed brains, and

have inferred that exposure to stressors, such as footshock, forced swimming, or conditioned fear, causes occupation of opioid receptors by endogenous ligands.[48-51]

Over the past few years, however, it has become increasingly clear that there are multiple subtypes of opioid receptors.[52] Since each of the previous stress and occupancy studies employed only a single, usually non-discriminating, radioactive ligand, we have conducted several studies to extend their findings by quantification of occupation of specific subtypes of opioid receptors following exposure to intermittent footshock stress or administration of morphine.[53,54] To measure occupancy of specific receptor subtypes, we have used two approaches. First, using subcutaneous injections of [^{3}H]naloxone or [^{3}H]etorphine to label mu receptors *in vivo*, we have shown that, compared to controls, rats exposed to intermittent footshock have decreased opiates bound in the brain. In fact, this decrease is comparable to that obtained by administration of a supra-analgesic dose of morphine (10 mg/kg). In a second series of experiments, we have coupled *in vitro* binding assays with *in vivo* manipulations. Rats were either subjected to intermittent footshock stress or served as non-stressed controls. Immediately after stress, brain homogenates were prepared and incubated with ^{3}H-labeled ligands of mu, delta, or kappa receptor selectivity. With this technique, footshock was found to cause an occupation of principally mu, but also delta and kappa receptors. These findings support the contention that exposure to stress can cause synaptic release of opioid peptides and suggest, as others have based on pharmacological data,[55] that mu receptors are particularly important in analgesic mechanisms.

COMPARISON OF CONTINUOUS AND INTERMITTENT FOOTSHOCK-INDUCED ANALGESIA TO OTHER FORMS OF STRESS ANALGESIA

Although it is often difficult to reconcile seemingly disparate findings between laboratories, parsimony would dictate that the numerous forms of stress analgesia characterized to date, ultimately will represent the activation of a finite number of analgesia systems. Toward this end, we have engaged in collaborative endeavors and conducted studies aimed at integration of findings between laboratories.

Importance of Stressor Intensity

As Terman *et al.*[17] have shown, the opioid or non-opioid basis of the analgesic response to continuous footshock stress is dependent upon the intensity and duration of the stimulus. This conclusion is similar to that presented by Fanselow[56] in studies of conditioned fear-induced analgesia. Moreover, Terman[18] has extended this intensity × duration principle to analgesia induced by another stressor, forced swimming. He has found that manipulation of the intensity of this stimulus (i.e. water temperature) can also determine the opioid or non-opioid nature of the resultant analgesia. Animals forced to swim in low temperature water display analgesia that is relatively refractory to naloxone, whereas the analgesic response to warm water swims is readily antagonized by this drug. This observation is consistent with those of Bodnar *et al.*[9] using cold water swims, and Christie *et al.*,[57] using warm water swims.

In 1982, Watkins and Mayer[30] published a series of studies suggesting that the body region shocked (i.e. front paws versus hind paws) can critically define the neurochemical basis of stress analgesia. We conducted an experiment to assess the role that stimulus intensity may play in their footshock paradigm.[58] We were able to reproduce their findings that shock applied to the front paws causes opioid-mediated analgesia and shocking the hind paws resulted in a non-opioid response. When the intensity of the stimulus was varied, however, opioid analgesia was obtained following low intensity shock, and non-opioid analgesia following higher intensities, independent of the body region shocked. These results suggest that in the work of Watkins and Mayer, in which similar current intensities were used to stimulate the front and hind paws, that footshock delivered to the hind paws had, for whatever reason, a sufficiently greater impact on the animal such that a non-opioid analgesia was evoked. These data are not meant to imply that the opioid and non-opioid forms of footshock analgesia elicited by continuous footshock are identical to those evoked by front and hind paw shock since other differences have been reported.[18,59] Nevertheless, it is our conclusion that shock intensity is a more critical determinant of stress analgesia neurochemistry than is the body region shocked.

Importance of Stress Controllability

A question that often arises is: What is different about intermittent versus continuous footshock that should lead to such dramatically different effects? Both of these stimuli are equal in intensity, are noxious as indicated by the animals' behavioral response, and both cause a nearly equivalent pituitary-adrenal stress response indexed by increases in plasma corticosterone.[60] The critical dimension on which they may vary is psychological. There is considerable evidence, beginning with the work of Maier and Seligman,[61] to suggest that equivalent amounts of stressful stimuli can have dramatically different impact on the animal depending upon whether or not the aversive stimulus is controllable.

The important role of stress controllability in the elicitation of stress analgesia has been demonstrated by Maier and colleagues. They showed that rats exposed to 80 inescapable tail-shocks, and tested 24 hr later following a reminder shock, displayed an opioid-mediated analgesia. Exposure to an equivalent amount of escapable shock, by contrast, was either without effect or caused a non-opioid analgesia.[62,63] This opioid-mediated form of analgesia shares several properties with that elicited by intermittent footshock; both forms are blocked by dexamethasone, hypophysectomy, and adrenalectomy.[29] Furthermore, if pain sensitivity is assessed immediately after the tail-shock procedure, 80 inescapable tail-shocks cause analgesia that is blocked by naltrexone, whereas 20 tail-shocks elicit analgesia insensitive to antagonist blockade.[64] This finding is quite parallel with our observations on the analgesic effects of intermittent and 4-5 minute continuous footshock, respectively.

Although all of the footshock stress procedures employed in our work are technically inescapable, it may be that only in the intermittent footshock condition do rats learn this contingency. To test this hypothesis, we engaged in a collaborative experiment with Maier and co-workers.[65] We showed that a single exposure to intermittent, but not continuous, footshock caused behavioral deficits in a shock-escape task. These deficits are termed "learned helplessness" and are similar to those disruptions induced by the inescapable, but not escapable, tail-

shock procedure used in Maier's work. Recently, we have confirmed and extended this observation by demonstrating that repeated exposure to intermittent, but not continuous, footshock causes behavioral deficits in a forced swimming model of "behavioral despair."[66,70] In further support of the contention that these opioid forms of analgesia are similar and dependent upon learning, are findings that both forms of analgesia are antagonized by scopolamine[24,67] and that this anti-cholinergic drug has previously been shown to disrupt learning, including learned helplessness.[68] Thus, taken together, these findings provided striking parallels between the analgesia due to intermittent footshock and that caused by inescapable shock, indicating that controllability or coping factors, and not simply exposure to stress per se, may dictate the impact of stressors on endogenous mechanisms of analgesia.

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