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Corticosterone: A Critical Factor in an Opioid Form of Stress-Induced Analgesia

Abstract. *The finding that some opioid-mediated forms of stress-induced analgesia are antagonized by hypophysectomy and dexamethasone has led to the suggestion that β-endorphin, released from the pituitary, may mediate these analgesic reactions. "Long-term analgesia" (an opioid-mediated form of stress-induced analgesia), which is blocked by dexamethasone and hypophysectomy, was also blocked by adrenalectomy and reinstated with corticosterone therapy. Corticosterone is proposed to play a permissive role in long-term analgesia and to be a critical hormone mediating this phenomenon.*

Exposure to a variety of stressors produces a subsequent decrease in pain responsiveness (*1*). This stress-induced analgesia has received considerable recent attention, largely because of its potential for providing insight into a possible functional role for endogenous opioids in behavioral and adaptive phenomena. The brain has pain-inhibiting systems in which endogenous opioids may play a role (*2*). The phenomenon of stress-induced analgesia suggested that endogenous opioids might be released by stress, thereby inhibiting pain and perhaps protecting the organism in some way (*3*). More recent work, however, has suggested that some forms of stress-induced analgesia are mediated by opioid systems [animals develop a cross-tolerance between the analgesic effects of morphine and the stress, and the analgesia can be reversed by opiate antagonists (*4*)], whereas others are mediated by nonopioid mechanisms (*5*).

Since multiple opioid systems exist in both the brain and the pituitary (*6*), recent research has been directed at determining which system mediates the opioid form of stress-induced analgesia. The discovery that hypophysectomy (removal of the pituitary) reduces an opiate-mediated stress-induced analgesia has supported the proposal that pituitary β-endorphin may mediate this type of analgesia (*1*). In further support of this view, the synthetic glucocorticoid dexamethasone has been shown to block both the stress-induced rise in plasma β-endorphin (*7*) and opioid stress-induced analgesia (*5*).

These and other findings led Baizman *et al.* (*8*) and Lewis *et al.* (*5*) to propose that pituitary β-endorphin might be mobilized by stress transported to the brain by retrograde flow through the portal system, and thus decrease responsiveness to painful stimuli by interacting with central structures. Such an action by pituitary β-endorphin is possible, but difficult to reconcile with reports that the hypothalamic content of β-endorphin decreases rather than increases after 30 minutes of footshock, and that even large doses of intravenous β-endorphin have little effect on pain responsiveness (*9*). Both hypophysectomy and dexamethasone treatment either eliminate or reduce the stress-induced release of pituitary adrenocorticotropic hormone (ACTH) as well as β-endorphin (*7*). This fact is noteworthy because corticosterone is under anterior lobe ACTH regulation, and corticosterone affects central processes associated with pain inhibition (*10, 11*). This line of reasoning suggests that the manipulations which seem to implicate pituitary β-endorphin may actually produce their effects by altering pituitary-adrenocortical interaction.

The purpose of our studies was to examine the role of the pituitary-adrenal axis in the production of the opioid form of stress-induced analgesia. A number of different procedures result in an opioid stress-induced analgesia. We used a procedure in which subjects are not tested until 24 hours after the stress session, but the pain responsivity test is preceded by a brief reinstatement procedure in which the subject is again exposed to the stressor (*4*). This allows for the dissipation of nonspecific factors such as fatigue and local anesthetic effects and may result in a "purer" opioid form of analgesia. The long-term effect is completely reversed by opiate antagonists and completely cross-tolerant with morphine, whereas these outcomes are not always complete under short-term testing soon after the stress session, even if the stress session is prolonged (*5*).

We first examined the effects of hypophysectomy on long-term analgesia. Eight hypophysectomized rats and eight that had been subjected to sham surgery (*12*) were restrained and treated with 80 5-second 1-mA shocks delivered through fixed tail electrodes on the average of one per minute. Eight more rats of each type were restrained for an equivalent period, but not shocked. Twenty-four hours later all rats received a shock reexposure procedure which consisted of five single-crossing shuttlebox escape trials. Immediately afterward, subjects were given three analgesia test trials (at 4-minute intervals) in a tail-flick apparatus in which latency to flick the tail from radiant heat served as the measure of pain sensitivity. These procedures are described elsewhere (*4*). Hypophysectomy completely blocked the long-term analgesia effect (Fig. 1A). A 2 by 2 analysis of variance confirmed this conclusion by showing significant shock [$F(1, 28) = 13.24$, $P < .01$] and hypophysectomy [$F(1, 28) = 12.13$, $P < .01$] main effects, and, most important, a significant interaction of hypophysectomy with shock [$F(1, 28) = 10.66$, $P < .01$]. Newman-Keuls individual group comparisons ($\alpha = .05$) indicated that the inescapably shocked sham rats were more analgesic than the other three groups.

In a second experiment, a 2 by 4 factorial design was used with rats restrained or inescapably shocked 2 hours after receiving an intraperitoneal injection of dexamethasone [0 mg per kilogram of body weight (saline), 0.25 mg/kg, 0.5 mg/kg, or 1.0 mg/kg]. As in the first experiment, 24 hours later all rats were given five shuttlebox trials and three analgesia test trials.

Dexamethasone prevented the inescapable shock-induced analgesia (Fig. 1B), with blockage complete at 0.5 and 1.0 mg/kg. Newman-Keuls individual

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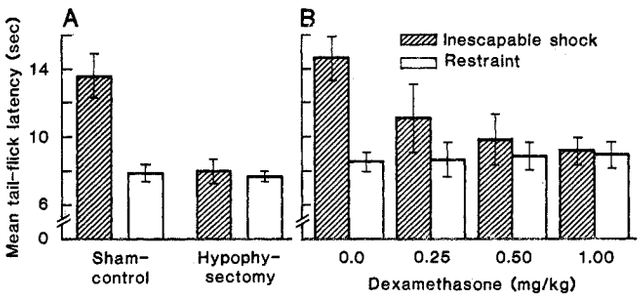

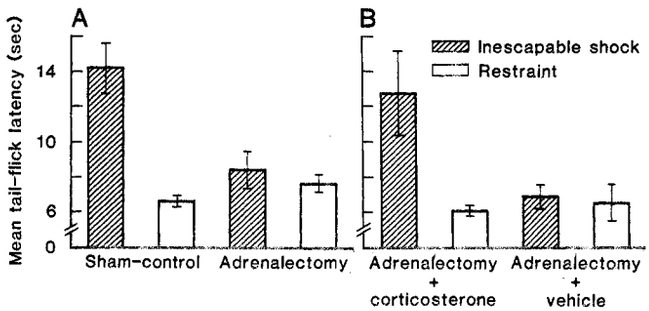

Fig. 1 (left). (A) Tail-flick latency for hypophysectomized and control subjects receiving either inescapable shock or restraint. (B) Tail-flick latency for subjects given either saline or different doses of dexamethasone before receiving either inescapable shock or restraint. Fig. 2 (right). (A) Tail-flick latency for adrenalectomized and control subjects receiving either inescapable shock or restraint. (B) Tail-flick latency for inescapably shocked and restrained, adrenalectomized rats receiving either corticosterone or the vehicle before reinstating shock.

group comparisons ($\alpha = .05$) indicated that the inescapably shocked group injected with saline was significantly more analgesic than all other groups except the inescapably shocked group injected with the lowest dose (0.25 mg/kg).

Hypophysectomy and dexamethasone blockade implicate the anterior pituitary in the production of the long-term analgesia, but these observations do not resolve the relative importance of ACTH and β-endorphin. If ACTH is critical and if its role is to induce the release of corticosterone, adrenalectomy should block the analgesic reaction. This is a strong test because adrenalectomy results in an elevation of plasma concentrations of β-endorphin (*13*).

Sixteen sham and sixteen adrenalectomized rats (350 to 400 g) were subjects (*12*). Half of the rats were inescapably shocked, the remaining rats were restrained, and 24 hours later all rats received five shuttlebox shock escape trials before three tail-flick tests.

Adrenalectomy completely blocked the long-term analgesia (Fig. 2A). A 2 by 2 analysis of variance revealed significant main effects of shock [$F(1, 28) = 23.10$, $P < .01$] and adrenalectomy [$F(1, 28) = 7.25$, $P < .05$] and, most important, a significant adrenalectomy by shock interaction [$F(1, 28) = 15.18$, $P < .01$]. Newman-Keuls post hoc comparisons ($\alpha = .05$) indicated that the inescapably shocked sham group was more analgesic than the other three groups. These data are consistent with the idea that corticosterone plays a critical role in the production of long-term analgesia. The data also suggest that β-endorphin is not critical, since the inescapably shocked adrenalectomized rats were not analgesic even though adrenalectomy elevates plasma concentrations of β-endorphin. However, the chronically high concentrations of β-endorphin may have desensitized or induced a tolerance in critical opioid processes (*14*), thereby blocking analgesia. If adrenalectomy blocks the long-term analgesia by removing circulating corticosterone, replacing corticosterone in adrenalectomized subjects might be expected to reinstate the analgesic reaction.

Adrenalectomized rats were either inescapably shocked ($N = 22$) or restrained ($N = 22$); half of each group received a subcutaneous injection of corticosterone (0.75 mg/kg), and the other half received the appropriate vehicle 6 minutes before the reinstating shuttlebox escape–tail-flick testing procedure. Total time from injection to tail-flick testing was 15 minutes.

Corticosterone reinstated the analgesia that had been blocked by adrenalectomy (Fig. 2B). A 2 by 2 analysis of variance confirmed the existence of a shock main effect [$F(1, 40) = 21.44$, $P < .01$], a corticosterone main effect [$F(1, 40) = 5.59$, $P < .05$], and a drug by shock interaction [$F(1, 40) = 14.37$, $P < .01$]. Subsequent Newman-Keuls comparisons ($\alpha = .05$) showed that the inescapably shocked group that received corticosterone was significantly more analgesic than the other three groups.

The results have relevance to the processes mediating short-term as well as long-term opioid stress-induced analgesia. The observations that hypophysectomy and dexamethasone treatment prevented the long-term stress-induced analgesia add to the previous opiate antagonist and cross-tolerance results in strongly suggesting that the long- and short-term forms are mediated by the same underlying neural processes.

Our results suggest that opioid stress-induced analgesia may not be mediated by pituitary β-endorphin. In addition, the results indicate that corticosterone plays a critical role in producing opioid-mediated stress analgesia. Since corticosterone replacement was administered only before testing (24 hours after the inescapable shock session) and still restored the analgesic reaction, it would seem that corticosterone is not required before, during, or soon after the inescapable shock. One might conclude, therefore, that some system is sensitized during exposure to inescapable shock and that corticosterone or one of its active metabolites plays a later permissive role in interacting with this system to inhibit pain. An obvious candidate is the midbrain system traditionally implicated in both opiate and stimulation-produced analgesia (*2*). Activating this system by opiates or electrical stimulation inhibits pain via descending serotonergic tracts terminating in the dorsal horn of the spinal cord (*2, 15*). Manipulations facilitating serotonergic transmission enhance both opiate and stimulation-produced analgesia, whereas manipulations interfering with serotonin-mediated transmission reduce both these and stress-induced analgesias (*15, 16*). Corticosterone is necessary for the stress-induced activation of tryptophan hydroxylase activity (*10*), the rate-limiting step in the synthesis of serotonin. This issue is relevant since dosages of corticosterone comparable to those we used activate serotonergic processes in midbrain structures (*11*).

Our results imply that central opioid systems may be involved in the mediation of stress-induced analgesia in two different ways: (i) in its traditionally hypothesized role in modulating the above-mentioned midbrain pain inhibition system and (ii) in the regulation of corticosterone. Opioid-innervated central structures participate in the regulation of the pituitary adrenal axis. For example, enkephalin-sensitive receptors in the hypothalamus aid in regulating corticotropin-releasing hormone (*17*), which in turn controls the activity of the pituitary-adrenal axis and thus corticosterone. This enkephalin-stimulated activation of pituitary-adrenal activity is blocked by opiate antagonists. Further, stress-in-

duced elevations of corticosterone are accentuated by synthetic opiate agonists and enkephalin administration and blocked by opiate antagonists (*17, 18*). Chronic morphine administration, as in cross-tolerance experiments, seems to decrease pituitary-adrenal function (*17*). Thus, opiate agonists and antagonists may exert some of their influence on opioid stress-induced analgesia by ultimately regulating corticosterone through a direct effect on enkephalin-sensitive opiate receptors, as well as through action on midbrain pain pathways.

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Widespread Periodic Intrinsic Connections in the Tree Shrew Visual Cortex

Abstract. *Intrinsic connections within the tree shrew* (Tupaia glis) *visual cortex (area 17) are organized in periodic stripelike patterns within layers I, II, and III. This anatomical network resembles the regularly organized stripes of 2-deoxyglucose accumulation seen after stimulation of alert animals with uniformly oriented lines. Such connections imply that widespread lateral interactions are superimposed on the retinotopic organization of area 17 and suggest alternative interpretations of cortical columns.*

A vertical or columnar organization of neocortex (*1*) has been suggested to underlie analysis of separate aspects of sensation (*2, 3*). In the primate visual cortex, this vertical organization is exemplified by two systems: ocular dominance and orientation specificity (*3*). Physiological recording (*4*) and 2-deoxyglucose (*5*) experiments appear to demonstrate columns, but there are still many questions about their structure and function. In particular, there is no well-defined anatomical basis for a cortical column extending through layers I to VI. Only in the instance of ocular dominance has an anatomical substrate been detected. The segregation of thalamic fibers according to ocularity has been clearly visualized in layer IV of the macaque (*6*), and it is then presumed that this segregation is reflected onto the adjoining layers, thus leading to a column influenced largely by one eye (*3, 6*). No anatomical basis has yet been delineated for the columnar organization of orientation specificity.

Reports of regularly arranged peaks of cytochrome oxidase or glutamic acid decarboxylase activity within layers II and III suggest even greater complexities in cortical organization (*7, 8*). The metabolically labeled patches appear to coincide with one another and can be related to patterns of ocular dominance and orientation specificity, as shown by accumulations of deoxyglucose. These findings suggest that the supragranular layers (II and III) have an intrinsic periodicity which must be taken into account in discussing the vertical organization of the cortex.

We recently demonstrated that the tree shrew (*Tupaia glis*) has a system of periodic intracortical connections within the supragranular layers which links widespread regions of primary visual cortex. Tree shrews, like monkeys, have a highly developed sense of vision and a complexly laminated visual cortex. Unlike the macaque, there is no physiological or anatomical evidence of ocular dominance domains in tree shrews (*9, 10*). However, deoxyglucose experiments reveal regularly spaced columns of high glucose uptake following visual stimulation of alert animals with lines of uniform orientation (*11*). The labeled columns form parallel slablike arrays that are aligned in a pattern resembling the physiologically mapped bands of orientation-specific neurons (*12*).

To demonstrate these intrinsic cortical connections we injected small volumes of horseradish peroxidase (HRP) (0.02 μl of 20 percent aqueous Boehringer HRP) in the primary visual cortex of seven tree shrews; in most cases the injection did not involve the underlying white matter. Bilateral injections were made in four animals. After 42 to 48 hours, the animals were anesthetized and perfused with mixed aldehydes followed by a sucrose buffer wash. The brain sections (30 μm) were reacted with tetramethyl benzidine (TMB) or diaminobenzidine chromogens. Effective injection sites—areas where dense reaction product obscured both neurons and neuropil—were estimated to be 0.5 to 1.2 mm in diameter.

Figure 1A shows an HRP injection in striate cortex; periodic densities of transported label extend for 2 to 3 mm on all sides of the injection site, and patches can be mapped over a distance of about 8

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