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Interaction of Acute and Chronic Stress with Respiration: Modification by Naloxone

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ISOM, G. E. AND R. M. ELSHOWIHY. *Interaction of acute and chronic stress with respiration: Modification by naloxone.* PHARMAC. BIOCHEM. BEHAV. 16(4) 599–603, 1982.—Rats exposed to inescapable foot shock displayed an increase in respiratory rate, tidal volume and minute volume. Naloxone HCl (5 mg/kg, SC) potentiated the foot shock-induced increase in ventilation. Inhalation of high (5% and 10%) concentrations of carbon dioxide enhanced the stimulation of ventilation observed in both the acute stressed animals and the acute stress-naloxone treated group. Chronic daily foot shock sessions (11 days) attenuated the respiratory stimulation produced by acute foot shock and the potentiation induced by naloxone. The appearance of foot shock-induced stimulation of respiration paralleled the production of acute foot shock-induced analgesia. On the other hand, chronic foot shock attenuated both stress-related analgesia and respiratory stimulation. These results strongly suggest stress can influence respiratory function through activation or release of the endogenous opioids. It is postulated that the endorphinergic system functions as a compensatory system which prevents excessive stimulation of respiration by stress.

Acute stress Chronic stress Respiration Naloxone Endorphins Rats

ENDOGENOUS opioid-like peptides, generically termed endorphins, function as inhibitory neuromodulators within the central nervous system and may participate in the regulation of a number of physiological systems [13,21]. It is becoming increasingly evident that endorphins modulate central respiratory function. Endorphins and opiate receptors are found within the pontine and medullary sites classically associated with respiratory regulation [2, 3, 5]. Following administration into the central nervous system, the peptides produce a stereospecific, dose-related depression of respiration and desensitize the central chemoreceptors to carbon dioxide [11, 12, 20, 27]. Recently, the low affinity opiate receptor has been identified as the mediator of opiate-induced respiratory depression [19, 22, 26]. It has been speculated that endogenous opioids produce a tonic inhibition of respiration through constant modulation of central chemoception [17,23]. In support of this concept, administration of naloxone, a specific opiate antagonist, stimulates respiration reportedly by displacing the endorphins from opiate receptor sites [7, 15, 17]. Also, naloxone increases phrenic nerve discharge [17] and appears to sensitize the central chemoreceptors to carbon dioxide [15].

In addition to serving as potential neuromodulators of physiological and behavioral processes, these endogenous agents may play an important role in the response to stress associated with certain pathophysiological systems. Stress is known to activate the endorphinergic system through mobilization of peptides from the pituitary and is accompanied by increased plasma and brain levels of endorphin [1, 4, 18]. Acute stress influences nociception and thermoregulation as a result of endorphin release and mobilization [21]. With regards to respiration, neonate hypoxia induces the release of endorphins and subsequently produces a depression of respiration and metabolism [8,14]. In chronic obstructive pulmonary disease, the release of endorphins and the accompanying changes in central respiratory function appear to constitute a compensatory response to the chronic stress of the disease process [24].

Even though behavioral stress appears to coincide with the occurrence of a number of pathological conditions of the respiratory system, the contribution of stress to the manifestations of the diseases is unknown. The present study was initiated to elucidate the interaction of acute and chronic stress with the central regulation of respiration and to correlate functional changes in respiration with the activity of the endorphin system.

METHOD

Respiratory function was determined in the unanesthetized rat using the whole body plethysmograph method [16], which entails monitoring respiratory rate, tidal volume and minute volume continuously throughout the experiment. Male, Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 120-180 gm were used in all studies. The animals were housed in com-

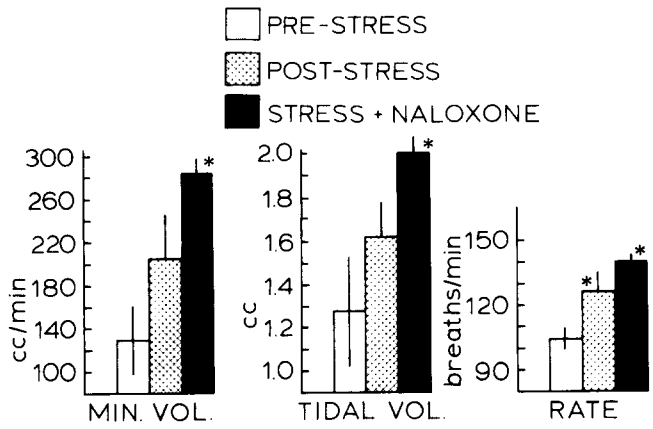

FIG. 1. Effect of 30 min stress session on the respiratory functions of rats breathing 95% 0_2-5% CO_2. Respiration was measured for 30 min before (pre-stress), and 5 min after the conclusion of the stress session (post-stress), and 5 min after administration of naloxone HCl (5 mg/kg, SC) to stressed rats. Respiratory rate is expressed as breaths per minute, tidal volume in ml and minute volume as total ml per minute. Each value represents the mean of 8 animals±SEM. Asterisks indicate significant difference from pre-stress value ($p<0.05$).

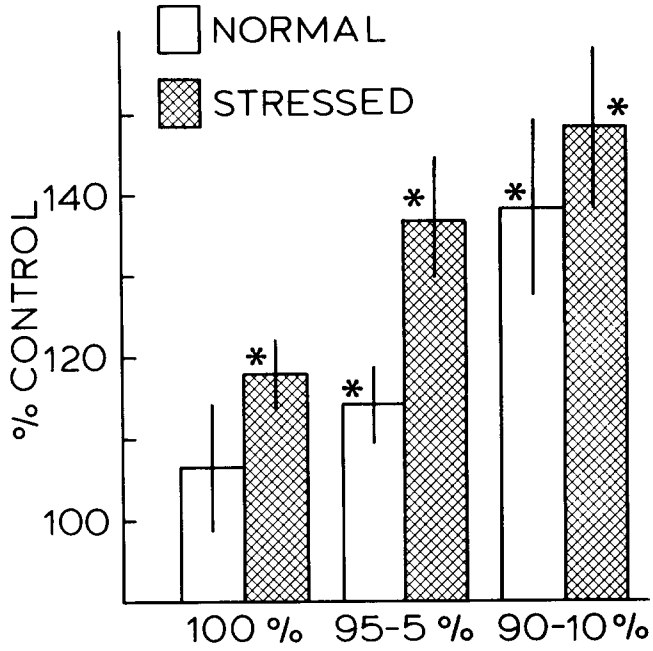

FIG. 2. Effects of varying the atmospheric carbon dioxide concentration on minute volume measured 5 min after the administration of naloxone HCl (5 mg/kg, SC) to normal or stressed rats. The inhaled gas composition was 100% O_2, 95% O_2–5% CO_2, or 90% O_2–10% CO_2. Each animal served as its own control and minute volume is expressed as percent of predrug control value. Each value represent the mean of 8 animals±SEM. Asterisks indicate significant difference from predrug control value ($p<0.05$).

munity cages containing 5–10 rats per cage and were maintained on standard laboratory diet and tap water ad lib. Each animal served as its own control throughout all experiments and respiratory parameters were expressed as percent control or as the absolute value.

In order to assess the interaction of stress, respiration was measured over a 30 minute recording period before and immediately after a 30 minute stress session. Pre-stress respiratory parameters served as control values and the plethysmograph atmosphere was maintained at 95% O_2-5% CO_2. In order to determine the role of the endorphins as possible mediators of stress, the action of naloxone on respiration was assessed in three experimental groups: (1) normal rats, (2) acute stressed rats and (3) chronic stressed animals. Naloxone HCl (5 mg/kg) was administered subcutaneous via an indwelling cannula to stressed animals (acute and chronic) 7 minutes post control period. Respiration was monitored for 30 minutes following naloxone administration. Depending on the experiment, the atmospheric concentration of carbon dioxide was varied from 0 to 10% with the balance being oxygen. Control groups underwent stress sessions but received normal saline injections (0.5 ml, SC) in place of naloxone.

Stress was induced by a 30 minute period of intermittent, inescapable foot shock. During each session, rats were placed individually on the grid of an operant chamber (LeHigh Valley Electronics, Beltsville, MD) and a 2 mA shock stimulus was delivered for 1 second duration at a frequency of every 10 seconds. Chronic stress was induced by exposing the animals to a 30 minute stress session, once daily, for a total of 11 consecutive sessions. Immediately following the stress session on day 11, respiration of each animal was monitored.

Pain responsiveness was determined by a modification of the classic tail-flick procedure of D'Amour and Smith [10]. The tail-flick-latency was determined by a tail-flick apparatus (Model TF-6, Emdie Instruments Co., Louisa, VA) on which the radiant heat source was adjusted such that the majority of the animals responded between 3.0 to 3.5 seconds during control testing. A cut-off point of 6 seconds was imposed to prevent tissue damage. Analgesia was measured immediately following the induction of stress and every 5 minutes over a 30 minute post-stress period.

Each experimental group consisted of eight or more animals and respiratory function was expressed as percent of pre-drug or pre-stress control ±SEM. Significance between treatment and control groups was determined according to Dunnett's multiple comparison test in which a probability level of 0.5 was used for testing significant differences.

RESULTS

An acute stress session produced a significant ($p<0.05$) elevation of respiratory rate (121% of pre-stress control) when measured five minutes after conclusion of the stress period (Fig. 1). Tidal volume and minute volume were elevated, but the increases were not significantly different ($p<0.05$) from pre-stress values. At all times during the recording period, respiration exhibited a regular but rapid rhythm in the stressed rats. The respiratory stimulation was a long term effect since total ventilation remained elevated for the 30 minute recording period. In order to assess the involvement of endogenous endorphins in the stress

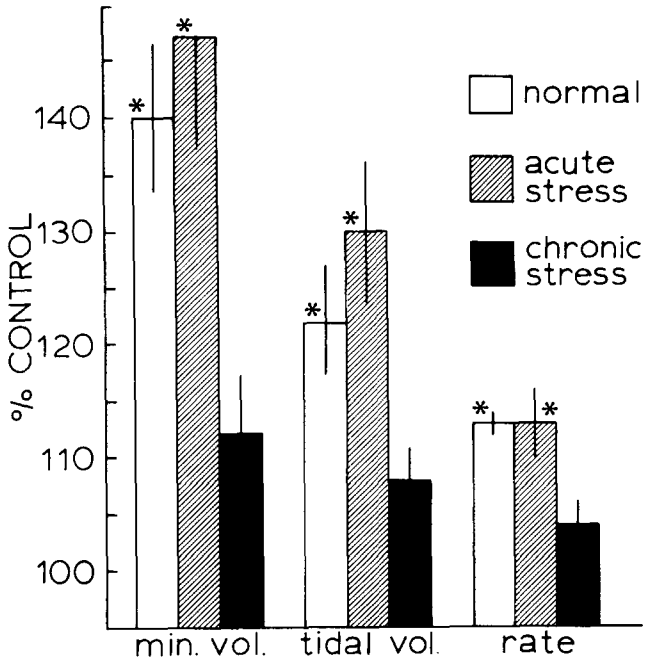

FIG. 3. Effects of naloxone HCl (5 mg/kg, SC) on respiratory functions expressed as percent of predrug control values in three different experimental groups. Group one (normal) did not undergo stress-induction; group two (acute stress) underwent a 30 min stress session prior to measurement of respiration; group three (chronic stress) underwent a 30 min stress session daily for 11 days and respiration was measured immediately following the last session. In all experiments the atmosphere was 90% O_2-10% CO_2 and the respiratory functions were monitored 5 min after administration of naloxone. Each value represents the mean of 8 or more animals ±SEM. Asterisks indicate significant difference from predrug control value ($p<0.05$).

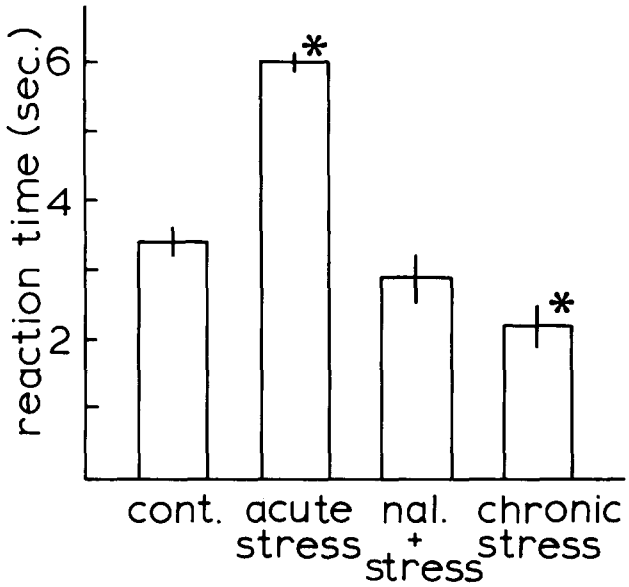

FIG. 4. Rat tail flick latency (seconds) in response to a thermal stimulus applied to the tail in various experimental groups. Normal animals (controls) received no treatments; acute stress group underwent a 30 min stress session immediately prior to measurement of tail flick; Naloxone + stress group was pretreated with naloxone (5 mg/kg, SC) 15 min before the stress session; and the chronic stress group received daily stress sessions for 11 days with nociception measured on day 11. Each value represents the mean of 8 or more animals ±SEM. Asterisks indicate significant difference from control value ($p<0.05$).

mediated changes in respiration, naloxone HCl (5 mg/kg, SC) was administered in a dose known to specifically antagonize opiate activity (Fig. 1). Naloxone potentiated the stress-induced stimulation of all three respiratory parameters.

In non-stressed animals, naloxone increased total ventilation in a step-wise manner as the CO_2 concentration inhaled by the rats was increased from 0% to 10% (Fig. 2). The changes in minute volume reflect similar increases in both tidal volume and rate (not shown). In acute stressed animals, naloxone increased ventilation to an even greater extent than that observed in non-stressed animals and the stimulation was carbon dioxide sensitive.

Chronic stressing of the animals attenuated the naloxone-induced increase of respiration in normal (nonstress) and acutely stressed animals (Fig. 3). All three respiratory parameters in naloxone treated rats were decreased after induction of chronic stress, as compared to normal (nonstressed) and acutely stressed rats. Following the final stress session on day 11, respiration was only slightly elevated (not significantly different from control, $p>0.05$) in both naloxone treated and non-drug treated animals (not shown) when compared to respiratory function on the first stress day. It is important to note that the magnitude of the naloxone-induced stimulation of respiration in chronic stressed animals was much less than that observed in nonstressed (normal) rats. Chronic stressing attenuated the stimulation of respiration observed after acute stress.

In order to correlate stress-induced changes in respiration with endogenous opioid activity, the response of various experimental groups to a thermal stimulus was studied. It is commonly accepted that as the levels of free endorphins within the brain increase, the tail-flick latency (pain threshold) will increase. Acute stress significantly ($p<0.05$) increased the tail-flick latency and naloxone (5 mg/kg, SC) pretreatment blocked the stress-induced analgesia (Fig. 4). As with respiration, chronic stressing of the animals attenuated the increase in tail-flick latency association with acute stress.

DISCUSSION

Endorphins appear to inhibit central respiratory regulation via a constant modulating effect on respiration by desensitizing the central chemoreceptors to carbon dioxide [27] and direct inhibition of respiratory neurons [11]. The

present study extends this concept by demonstrating acute foot shock can influence several parameters of respiration in the unanesthetized animal and the endorphins may modulate the response. It is conceivable that following foot shock, endogenous opioid-like peptides are released in the brain to inhibit respiration. In the case of respiration, the endorphins may function as a compensatory system which prevents excessive stimulation of respiration during stress.

It was observed that acute foot shock increased total ventilation and this appears to be in conflict with previous observations that stress releases and activates the endorphin system [18]. However, it is known that additional factors such as ACTH and the accompanying changes in metabolism and the adrenal catecholamines mediate stress responses and these factors are known to stimulate respiration [9,25]. Activation of these stimulatory agents may mask the depressant action of the endorphins. In support of this concept, naloxone induced a marked increase of respiration in acutely stressed animals. The narcotic antagonist appears to block the inhibitory action of the endorphins on respiration.

With chronic foot shock both the stress-induced stimulation of respiration and the interaction of naloxone with stress were attenuated. Pituitary levels of ACTH and endorphins are known to be depleted by chronic stress [1, 4, 18], and in turn, the influence of both ACTH and endorphins on respiration would be attenuated as observed in the present experiment. Since stress-induced analgesia may be mediated by endorphins released from the pituitary gland, changes in nociception should correlate with endorphin mediated changes in respiration. The stress-induced analgesia was naloxone sensitive indicating the involvement of endogenous opioids. Chronic stress reversed both antinociception and respiratory responses suggesting depletion of endorphins as previously reported [18]. Also, the release of endorphins following each stress session over the 11 day treatment period may lead to tolerance to the respiratory depressant effects. Tolerance to the respiratory depressant action of the endorphins has been observed [27].

The naloxone-induced stimulation of respiration observed in both stressed and normal animals increased in a step-wise manner as the atmospheric carbon dioxide was increased. Since the response of the two different experimental groups was parallel, it is conceivable a common underlying mechanism of action of naloxone exists. In normal animals naloxone blocks the tonic inhibition of respiration produced by the endorphins, resulting in an increased responsiveness to carbon dioxide and an increase in respiratory exchange [15]. In stressed animals, naloxone would block the endorphin inhibitory modulation and respiration would be increased to a greater extent than in the normal animals since stimulatory stress factors would be activated.

Previous studies suggest the endorphins comprise an important component of the respiratory response to stress. Belenky and Holaday [6] reported that pretreatment of rats with naloxone produced an increase in respiratory rate following electroconvulsive shock. Recently, Santiago *et al.* [24] observed that naloxone restored flow-resistive load compensation in patients with chronic obstructive pulmonary disease. These findings suggest the activation of the endorphinergic system is a mechanism by which an individual may adapt to the stress of chronic airway obstruction.

In conclusion the present study suggests stress can influence respiration through release of endorphins. Additional study is needed to delineate the pathophysiological significance of the endorphins in respiratory regulation, particularly under stressful conditions.

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