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Short communication

Alpha-melanocyte stimulating hormone reduces putative stress-induced sickness behaviors in isolated guinea pig pups

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Abstract

We have proposed that passive responses observed following maternal separation in guinea pig pups represent "stress-induced sickness behaviors" mediated by proinflammatory processes. In this study, the anti-inflammatory peptide, alpha-melanocyte stimulating hormone (α-MSH) administered intracerebroventricularly, but not intraperitoneally, reduced the passive responses of crouching, eye-closing, and extensive piloerection relative to levels following administration of vehicle. These findings support our hypothesis and are as would be expected if pro-inflammatory processes act centrally to promote the passive behaviors of separated guinea pig pups.

Keywords: Alpha-melanocyte stimulating hormone; Stress-induced sickness; Passive responses; Guinea pig; Maternal attachment; Sickness behavior

From the time of Harlow's [10] initial work, studies of behavioral and physiological responses during maternal separation or isolation have played a central role in our understanding of the development of emotional responsiveness and psychopathology. The early observation that some species of monkeys exhibited a two-stage, active/passive response to separation procedures that resembled the so-called "protest" and "despair" stages previously described in institutionalized children established the separation paradigm as an animal model for early emotional dysfunction [22,43]. Moreover, current theories of the role of early stress in the ontogeny of depression have relied heavily on studies of maternal separation in laboratory rats and mice [e.g., 12]. Although much has been learned from work with rats and mice, they are of limited value for some purposes because they do not show the same kind of specific emotional attachment to the rearing figure as do most primates, including humans. The guinea pig, which displays clearer evidence for filial attachment, appears to be a useful alternative. Many similarities have been documented in the reaction to separation in guinea pig pups and primate infants [13]. Among these is a two-stage, active/passive response during maternal separation and isolation in a novel environment [17]. Recently [15,16], we have suggested that the passive responses, consisting of reduced vocalizing and locomotor activity, together with a characteristic crouched stance, eye-closing, and extensive piloerection, may represent "stress-induced sickness behaviors."

Sickness behaviors refer to a constellation of behavioral changes produced as a part of the acute phase response of the immune system, which characteristically occurs in reaction to infection [2,23]. Examples of sickness responses to infection include reduced social, sexual and locomotor activity, together with increased sleepiness, shivering, piloerection, and the assumption of hunched postures [1,11,37]. These behavioral changes are all thought to facilitate adaptive recovery from the invading pathogen by promoting fever and conserving resources necessary for other energetically expensive processes such as immune cell proliferation [11]. At the mechanistic level, many sickness responses appear to be governed by pro-inflammatory cell-derived products such as cytokines, chemokines, and prostaglandins acting in the central nervous system.

It is now recognized that stressors can frequently induce elements of the acute phase response, including sickness behaviors [29]. Indeed, there are several converging lines of evidence to indicate that pro-inflammatory factors play a role

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in the precipitation of behavioral responses to stressors. For instance, exposure to stressors has been shown to produce fever [24,33,36], changes in circulating acute phase proteins [7], and increased expression of cytokines in both brain [34,35] and blood [35]. Futhermore, inhibition of pro-inflammatory cytokine activity in the CNS has been shown to block impairments in memory consolidation produced by social isolation [39], interfere with learned helplessness produced by inescapable shock [28], blunt the hypothalamic monoamine response to immobilization [42], and reverse reserpine-induced immobility in the forced swim test [33]. Moreover, immune activation produces a very similar complement of behavioral [29], neuroendocrine, and neurochemical [44] changes as those observed following stressor exposure. Together, these findings suggest that pro-inflammatory cytokines and possibly other related immune factors are involved in the production of behavioral responses incurred by stressor exposure.

In guinea pig pups, we found that administration of lipopolysaccharide (LPS), a potent inducer of the acute phase response, reduced vocalizations and locomotor activity, and increased crouching, eye-closing, and piloerection relative to vehicle-treated control pups [16]. This is the same profile of behavioral changes observed over the course of a several hour separation of pups from their mother [17]. If the behavioral effects of isolation in guinea pig pups do, in fact, represent stress-induced behaviors mediated by a pro-inflammatory process, then it should be possible to prevent or reduce these responses by administering an agent with broad anti-inflammatory properties. One such agent is alpha-melanocyte stimulating hormone [α-MSH; 5, 25]. Intracerebroventricular (ICV) administration of α-MSH can reverse various responses associated with sickness, whether sickness is induced by pathogen exposure or stress. Responses reversed by ICV α-MSH include: fever [21,37], the release of various proinflammatory cytokines [25,40], increased HPA activity [27,31] and aphagia/adipsia [32]. Anti-inflammatory effects of α-MSH have been observed in various species, including guinea pigs [21]. Therefore, in the present study, we examined whether ICV α-MSH would reverse the passive responses of isolated guinea pig pups.

Albino guinea pigs (*Cavia porcellus*) were bred in our laboratory. Each mother and its litter were housed in plastic cages (73 cm × 54 cm × 24 cm) with sawdust bedding. Water and guinea pig chow were available ad libitum. Lights were maintained on a 12:12 light/dark cycle with lights on at 7:00 a.m. Ambient temperature in the colony and testing rooms was maintained between 22 and 25 °C. All procedures were approved by the Wright State University Laboratory Animal Care and Use Committee.

Pups underwent surgery for placement of a cannula aimed at the right lateral ventricle between days 16 and 19 (with the day of birth considered day 0) under diazepam (9.0 mg/kg)/sodium pentobarbital (23 mg/kg) anesthesia with additional local anesthesia to the scalp (0.25 mg/0.1 ml 0.25% bupivicaine). Guide cannulae (26 gauge) were placed relative to bregma with coordinates of −3.0 mm anterior-posterior, −3.0 mm lateral, and −4.0 mm dorsal–ventral, and a stainless steel screw was placed adjacent to the guide cannulae to help secure the cranioplastic cement. All cannula supplies were sterile at the time of surgery and were purchased from Plastics One (Roanoke, VA). All pups were treated with buprenorphine (0.015 mg/0.05 ml) post-surgery to control for post-operative pain. Each day, animals were weighed and dummy cannulae were checked for patency. Animals were allowed to recover from surgery for at least 3 days [8,26] prior to the first test. All animals were killed after the second test via carbon dioxide inhalation. Cannulae placement was then verified via dye infusion; only data from animals in which dye was present in at least one lateral were included.

Pups ($n = 9$, three males, six females) were tested once between days 19 and 22 and again between days 22 and 25, with at least 4 and at most 6 days between tests. During this developmental period, pups exhibit a robust response to separation from their mothers [19]; pups are weaned at day 25 in this laboratory. Pups were manually infused using a sterile 33 gauge internal cannula attached to flexible tubing and a glass Hamilton microsyringe (1 μl/30 s) with either α-MSH (dissolved in 5 μl saline) or the saline alone. Conditions were presented in a counterbalanced order. The dose chosen for this initial study was 25 μg, which on a body weight basis is in the upper range of doses found to be effective in rodents when administered ICV [5,21]; pups weighed approximately 250–300 g on the 1st day of testing. After dosing, pups were placed back into their home cage with their mother and any littermates for 60 min. For testing, pups were transported into a test room that adjoined the colony room. Pups were then placed alone in a clean, clear plastic cage (47 cm × 24 cm × 20 cm) under full indoor illumination. The test cage was thoroughly washed with water and detergent between tests. Pups were observed over the course of 180 min, with data being collected at intervals 0–30, 60–90, and 150–180. The effects of ICV α-MSH have been shown to last for at least four hours [45]. A trained observer, who was not blind as to the condition of the pups, scored behavioral events while sitting behind a one-way glass. A single observer was used for both conditions per pup. Both active behaviors (whistle vocalizations [4] and line crossings—the number of times pups crossed lines on the cage floor that divided it into four equal sections) and the passive responses characteristic of prolonged isolation: crouch (body hunched down with the head lowered and feet tucked beneath the body), eye-close (one or both eyes completely or nearly completely closed for more than 1 s), and piloerection (occurring over most of the visible body surface) were recorded. Because single instances of crouch, eye-close, and piloerection typically occur over an extended period of time, these behaviors were scored with one-zero sampling as in previous studies [16]. Specifically, we noted whether the behavior occurred during any portion of consecutive 60 s intervals. Vocalizations were scored with a hand-held counter; other behaviors were scored with pencil and paper. Interobserver reliability was 85% or above within each behavioral category.

To assess whether any observed effects of α-MSH were indeed due to central, rather than peripheral actions (e.g., due to leakage into circulation), an additional 10 pups (four males, six females) were tested using the exact test procedures as described