Short communication

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

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Received 21 April 2005; received in revised form 23 August 2005; accepted 26 August 2005
Available online 7 October 2005

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
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]. Furthermore, 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% bupivacaine). 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 cranioplast.
above with the exception that they were unoperated and 25 μg of α-MSH or saline was administered i.p. 60 min prior to testing. Data were not normally distributed and scores of “zero” were common; therefore, the total incidence of behavior (summed across intervals) was analyzed using non-parametric tests. Males and female exhibited similar patterns of responding, thus their data were pooled for analysis. Wilcoxon paired comparisons were used to assess whether behavior differed between the saline and α-MSH conditions. A two-tailed significance level of 0.05 was used.

As can be seen in Fig. 1, there was a tendency for ICV α-MSH to increase vocalizations and line crossings, but this was not statistically significant. Although median numbers of these behaviors were much higher in the α-MSH condition, only 4/9 pups showed an increase in vocalizations, and 5/9 exhibited an increase in the number of line crossings, when treated with α-MSH. Consistent with our hypothesis, central administration of α-MSH did reduce the number of intervals during which pups displayed the crouched posture, p < 0.02, eye-closings, p = 0.05, and extensive piloerectretion, p < 0.05. For crouching and piloerection, 8/9 pups exhibited reductions following α-MSH treatment relative to controls, for eye-closing 7/9 did so. Administration of α-MSH peripherally produced no significant changes in any behavioral measure.

Because the behavioral responses to prolonged maternal separation of guinea pig pups resemble behavioral responses produced by peripheral injection of LPS [16], we predicted that the expression of passive behaviors following isolation in a novel environment might be blocked by central administration of an anti-inflammatory agent. This prediction was confirmed in that ICV α-MSH significantly reduced the crouching, eye-closing, and piloerection produced by isolation in a novel environment.

The fact that the same dose of α-MSH injected peripherally failed to alter the behavioral responses indicates that the effects of ICV α-MSH were due to central actions of the peptide, rather than the drug leaking into the periphery following infusion. Together, these data suggest that behavioral responses to a several hour maternal separation may result, at least in part, from increased expression of pro-inflammatory related factors in the CNS, and provide support for our hypothesis that these responses may be viewed as stress-induced sickness behaviors [15]. This conclusion is bolstered by previous observations that the isolation procedure increases both core temperature [16] and hypothalamic-pituitary-adrenal activity [20], two physiological components of the sickness reaction.

Although α-MSH is a powerful anti-inflammatory agent, we cannot rule out the possibility that the peptide affected behavior through some other mechanism. However, additional support for the importance of the anti-inflammatory action of the compound derives from our recent unpublished observation that indomethacin, a blocker of prostaglandin synthesis, can also reverse the crouching of guinea pigs during social isolation. Further, while we do not know how the stress of isolation in a novel environment might lead to immune activation, our results to date suggest that the cytokine IL-1β is not involved [16], as central and peripheral levels of this cytokine were not observed to change following isolation. We do know, however, that the stress-reactive peptide corticotropin-releasing factor (CRF) can produce the same reduction in active, and increase in passive, behaviors as can LPS, or a several hour period of isolation in a novel environment [3,14,17]. Moreover, these effects are blocked by administration of CRF antagonists, and the antagonists alone have effects that are opposite of those of CRF [17,18,30]. The interactions between CRF and
the immune system are numerous. Immune factors, including cytokines, can activate CRF, and CRF can modulate the effects of the acute phase response [6]. The behavioral effects of prolonged isolation on passive behaviors could therefore be due to cytokine activation of CRF, or CRF activation of cytokine release. To begin to address this question, work currently underway in our laboratory is assessing whether α-MSH can block the effects of exogenous CRF.

In other species, administration of α-MSH has been found to produce apparent anxiogenic effects, increasing distress vocalizations in chicks [38] and reducing time spent in the open arms of an elevated maze in rats [9,41]. However, with the exception of a non-significant increase in vocalizations, we observed no behavior suggestive of increased anxiety following α-MSH infusion. While it is not clear why α-MSH might increase anxiety in some animal models and not others, differences in such factors as species, dosage, and test procedures are possible explanations.

The interpretation of the onset of passive behavior following prolonged maternal separation in primates has traditionally been interpreted as “despair” in part because of the similarity in behavior between separated non-human primates [22] and maternally deprived children [44]; both exhibit reduced activity, reduced social responsivity, lethargy, aphagia, and the assumption of species-specific postures, such as a huddled posture in macaques [22] or prolonged lying face down in children [43]. These changes are at least superficially similar to what we have described in maternally separated guinea pig pups. Therefore, the present results suggest the possibility that sickness behaviors contribute to the “despair” stage in primates warrants further study.

Acknowledgements

This work was funded by National Institutes of Mental Health Grant MH68228. Tess M. Greenlee is now at the University of Indianapolis. The authors wish to thank Katie BuLLinger for help in data collection.

References


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