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Effects of Naloxone on Corticosterone Response to Stress

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TAPP, W. N., J. C. MITTLER AND B. H. NATELSON. *Effects of naloxone on corticosterone response to stress.* PHARMAC. BIOCHEM. BEHAV. 14(5) 749–751, 1981.—The effect of naloxone on the corticosterone response to restraint stress was examined. Naloxone (8 mg/kg, IP) did not alter basal corticosterone or the magnitude of the corticosterone response to restraint stress. Naloxone did, however, retard the fall in corticosterone following the end of restraint stress; thus the drug prolonged the stress response. These data suggest that endogenous opiates play a role in the restoration of corticosterone levels back to normal after stress.

Naloxone Corticosterone Stress

LARGE doses of the opiate antagonist naloxone have been reported to increase corticosteroid levels in man [8], mouse [3] and rat [1]. These findings have led to the hypothesis that endogenous opiates may exert an inhibitory influence on ACTH release [1,8]. In his discussion of this possibility, Eisenberg [1] suggested that if this explanation was valid, one might anticipate an increased corticosterone response to a stressor in a naloxone-pretreated rat. However, the only reported study which tested this possibility found that naloxone-treated mice exhibit declines in corticosteroids following exposure to ether "stress" [3]. Unfortunately, this experiment cannot be generalized easily because ether's well-known direct pharmacological effect on the median eminence-pituitary unit could alter or obscure the role of endogenous opioids in the stress response. Moreover, a recent review [7] suggests that naloxone may antagonize a variety of general anesthetics, so naloxone may have had direct pharmacological effects on the visceral response to ether. Thus it is possible that the findings of Gibson *et al.* [3] reflect some pharmacological interaction between naloxone ether. Therefore, we designed the present experiment to collect new data to address the issue of what naloxone-pretreatment would do to a rodent's corticosterone response to stress. Rather than use a pharmacological stressor such as ether, we used immobilization—a physical and psychological stressor. Since its early description by Selye [6], immobilization has been frequently used to produce visceral activation characteristic of the stress response (e.g., [4]).

METHOD

Male Sprague-Dawley rats (Taconic Farms) weighing between 250 g and 350 g were individually housed in 12 hr light–12 hr dark with ad lib access to food and water. Rats were implanted with superior vena caval catheters which extended outside the rats' cages. Catheters were protected from gnawing by springs. This method permits repeated blood sampling without disturbing the freely-moving rat.

Four days after the catheters were implanted, rats were randomly assigned to one of four groups (6 rats/group): Naloxone-Restraint (NR), Saline-Restraint (SR), Naloxone-Unrestrained (NU), Saline-Unrestrained (SU). After an initial pre-stress blood sample was collected, the rats were weighed and injected IP with either naloxone HCl (8 mg/kg dissolved in 1 ml/kg saline) or with 1 ml/kg of physiological saline. The IP route was used because Gibson *et al.* had used it in their experiments [3]. Rats in restraint groups were then restrained for one hour in the prone position by taping their limbs to the corners of a small board and by covering their bodies with a leather flap that was attached to the board with velcro. Rats in unrestrained groups were handled twice, once corresponding to the beginning of restraint and once corresponding to the end of restraint. Blood samples (0.8 ml each) were drawn after 15 min of restraint, after 60 min of restraint, 30 min after the end of restraint, and at the equivalent times in unrestrained animals. Heparinized plasma was separated by centrifugation and stored at −40°C. The packed cells were resuspended in saline and returned to the rat following plasmaphoresis.

Corticosterone Assay

Plasma corticosterone was measured by radioimmunoassay. Plasma samples were diluted tenfold in a borate buffer containing 5% gelatin (pH 8). Dilute plasma was heated at 70°C for 1 hr to denature binding proteins. An aliquot of dilute plasma was assayed for corticosterone using a corticosterone antibody (B21-42) from Endocrine Sciences (Tarzana, CA) and [^{3}H]-corticosterone (Amersham) was used as trace. Standard corticosterone was obtained from Sigma (St. Louis).

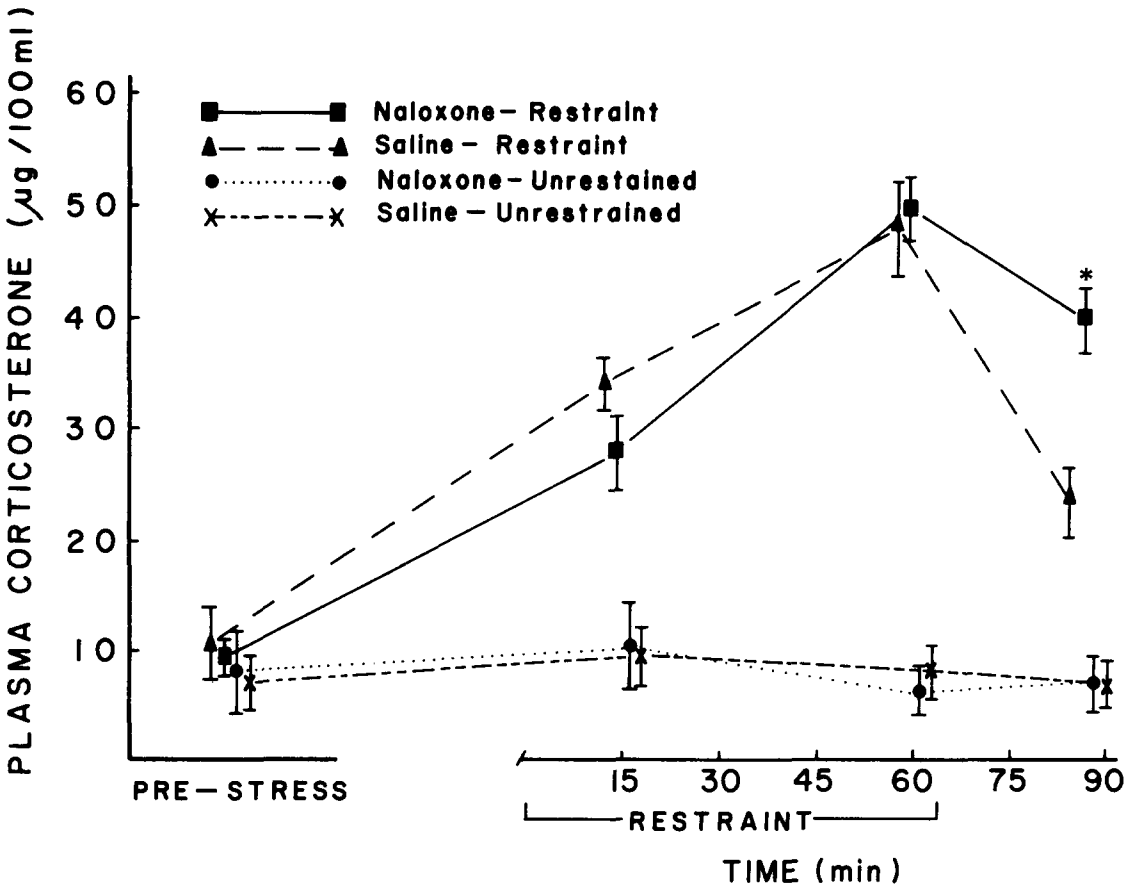

FIG. 1. Effects of naloxone on basal corticosterone levels and on the corticosterone response to restraint stress. Except for the pre-stress sample, all of the values for restrained animals are significantly higher than the values for unrestrained animals ($p<0.01$ by Tukey's test). Points illustrate the mean ± SEM. *NR significantly different from SR ($p<0.01$ by Tukey's test).

RESULTS

Results are shown in Fig. 1. Restraint significantly elevated plasma corticosterone, $F(1,20)=93.15$; $p<0.01$, and there were significant variations in corticosterone across time, $F(3,60)=49.21$, $p<0.01$. However, the significant Restraint Condition×Time of Sample interaction showed that only the restrained animals exhibited appreciable variations in corticostene, $F(3,60)=51.1$; $p<0.01$. Unrestrained animals exhibited levels in the basal range throughout the experiment. Importantly, naloxone did not increase plasma corticosterone in these unrestrained rats.

Naloxone did alter the pattern of the corticosterone stress response in restrained rats as revealed by the significant Drug Condition×Time of Sample Interaction, $F(3,60)=4.34$; $p<0.01$, and the significant Restraint Condition×Drug Condition×Time of Sample interaction, $F(3,60)=4.94$; $p<0.01$. Individual comparisons (Tukey's test) between the NR and SR groups showed that there were no differences in plasma corticosterone of naloxone- and saline-treated restrained rats prior to restraint, after 15 min of restraint, or after 60 min of restraint. However, 30 min after the end of restraint, plasma corticosterone was significantly higher in naloxone-treated rats than in saline-treated rats. Thus naloxone did not elevate basal corticosterone levels or increase the corticosterone response to stress, but naloxone retarded the fall in plasma corticosterone after the end of stress.

DISCUSSION

Our purpose in doing this experiment was to see the effect of naloxone on restraint stress-induced activation of the hypothalmo-pituitary-adrenal axis. Although we found no difference in the corticosterone response during the 30 min stress, we found that the drug retarded the fall of corticosterone following the end of stress. Thus, naloxone affected the duration but not the magnitude of the stress-induced corticosterone increase. These findings are in striking contrast to those of Gibson *et al.* [3] who reported lower levels of corticosterone in naloxone-pretreated mice compared to controls 15 min after a 1 min exposure to ether "stress". Although this difference may reflect a species difference, we believe that the observations of Gibson *et al.* reflect some pharmacological interaction between ether and naloxone at median eminence neurons or pituicytes. We believe these data lend credence to Eisenberg's hypothesis [1] that naloxone's effect at increasing plasma corticosterone [1, 3, 8] is due to its blocking endorphin receptors which inhibit

ACTH release. However, another possibility suggested by Eisenberg remains: That corticosterone increases because naloxone precipitates a withdrawal stress response to the "dependence" on endogenous opiates.

Our data mitigate against a third explanation proffered by Eisenberg [1], that of high doses, naloxone has a direct agonist effect on opiate receptors. Under the conditions used here, naloxone did not alter basal levels of corticosterone in unstressed rats but allowed an effect to be seen as the corticosterone response was recovering from the stress. This dissociation of effect on levels of corticosterone produced by 1 dose of naloxone minimizes the possibility of the mechanism being an agonistic action of the drug.

That naloxone did not increase levels of corticosterone in unstressed rats is different from earlier work [1]. However this is probably due to differences in the dose and route of administration of naloxone. Since IV doses of 10 mg/kg of naloxone quickly increased levels of corticosterone while 2 mg/kg did not, we assume that our dose of 8 mg/kg IP may have been beneath the threshold required to increase levels of the hormone in unstressed animals.

Thus an apparent paradox exists. While 8 mg/kg of naloxone was not sufficient to increase basal corticosterone levels in unstressed rats, it was sufficient 90 min later to retard the return of the stress-elevated corticosterone to resting levels. One possible explanation for this paradox is that there are two receptor populations involved in inhibitory control of corticosterone. The receptor population involved in maintaining basal levels is less sensitive to naloxone than the receptor population that is involved in return to basal levels following stress.

The other inference that can be made from this paradox relates to the duration of effect of the drug. Ngai *et al.* [5] have shown that more than 90% of an IV dose of naloxone is eliminated from the brain within the first hour; this suggests that the biological activity of the drug is brief. Our physiological data and those of Eisenberg and Sparber [2] suggest that this is not the case and that the drug can have an effect for well over an hour.

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