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Central But Not Peripheral Opiate Receptor Blockade Prolonged Pituitary-Adrenal Responses to Stress

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ODIO, M. AND A. BRODISH. *Central but not peripheral opiate receptor blockade prolonged pituitary-adrenal responses to stress.* PHARMACOL BIOCHEM BEHAV **35**(4) 963–969, 1990.—Evidence from pharmacological studies suggests that opiate systems may serve either inhibitory or stimulatory functions on stress-induced responses of the hypothalamic-pituitary-adrenocortical (HPA) axis. The objective of these experiments was to determine whether these discrepant findings may result, in part, from differential effects of central or peripheral opiate receptor blockade on HPA axis responses. To this effect, groups of rats received injections of either saline, naltrexone (NHCl) or the quaternary analogue naltrexone methobromide (NMBr). The animals were then exposed to 30 min of a motion stressor and blood samples were obtained from each rat for analysis of ACTH, corticosterone, and prolactin. The data showed that resting and stress-induced levels of prolactin were decreased by NHCl only. Although neither drug affected the magnitude of the stress-induced ACTH and corticosterone responses, treatment with NHCl, but not NMBr, delayed the poststress decline of these responses. Hence, we concluded that central opiate mechanisms may be important for cessation of HPA axis activity, after exposure to stressful situations.

Tertiary and quaternary naltrexone Stress-induced ACTH corticosterone and prolactin responses

PHARMACOLOGICAL approaches to elucidate the role of opiate systems on resting and stress-induced activity of the hypothalamic-pituitary-adrenocortical (HPA) axis have yielded conflicting results. For example, it was reported that the corticosterone response induced by exposure to immobilization, cold and ether stress was inhibited by administration of opiate receptor antagonists (9,11). These findings were consistent with other studies which found that opiate agonists such as morphine (4, 18, 21) and enkephalinamide (7) elicited adrenocorticotropin (ACTH) release and increased stress-induced plasma levels of ACTH and corticosterone and that these effects could be reversed by naloxone. However, other investigators have reported that naloxone pretreatment prolonged or enhanced the corticosterone response elicited by exposure to immobilization, photic and acoustic stress (25,31). Similarly, the role that opiate systems may have on the regulation of the HPA axis under basal conditions remains uncertain in view of reports which showed increased (8, 11, 18) or unaltered (9,31) resting levels of ACTH and corticosterone after administration of opiate receptor blockers.

The reasons for these different findings have not been fully resolved. In the case of resting levels of ACTH and corticosterone, an important factor appears to be the dose of antagonist that was administered since, in rats, low doses of naloxone had no effect (8, 9, 31), whereas higher doses of the compound increased resting levels of these hormones (8, 18, 25). With respect to stress-induced activation of the HPA axis, differences between stressors could explain some of the discrepant effects of opiate receptor blockers that have been reported. This possibility can be proposed based on results which suggest that specific stressors may differ in the degree to which they involve opiate receptor-mediated processes (32). Furthermore, it is now known that during stress, opioid peptides can be secreted from the adrenal medulla (15,34) and that these peptides can exert typical centrally mediated effects (6), but may also mediate peripheral actions such as localized analgesia (16) and modulation of adrenocortical responses to ACTH (2, 13, 22). Thus, discrepancies about the role of opiates in stress-induced activation of the HPA axis could also arise, in part, from the degree to which particular stressors involve a different balance of central and peripheral opiate actions.

In the present experiments, a tertiary and a quaternary amine derivative of the opiate receptor blocker naltrexone were used as a pharmacological approach to distinguish between centrally and peripherally mediated opiate effects on stress-induced HPA axis responses. The stressor used to elicit ACTH and corticosterone responses was a motion stimulus which we have characterized extensively in previous reports (3,19). This stressor was used because it involves virtually no tactile or nociceptive stimulation of the rats, which diminishes the possibility that drug-associated

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effects on HPA responses could be mediated indirectly by blockade of other opiate actions such as stress-induced analgesia. As a further means of confirming that, at the doses administered, the drugs used were exerting specific pharmacological actions at opiate receptor sites, resting and stress-induced prolactin levels were also measured in all rats. This was considered to be an appropriate control biomarker because there is substantial agreement in the literature that administration of opiate receptor antagonists decreases resting and stress-induced prolactin levels (24, 26, 33). In our experimental design resting levels of ACTH and corticosterone were unaffected by administration of the opiate receptor blockers at the dose that was used in these experiments. By contrast, stress-induced ACTH and corticosterone responses were prolonged by the centrally acting opiate receptor blocker and were unaffected by the quaternary derivative of naltrexone. In addition, the data confirmed earlier reports (24, 26, 33) that resting and stress-induced prolactin levels are decreased by opiate receptor antagonists, and further showed that this is a centrally mediated action since the effect was seen with the tertiary but not the quaternary amine derivative of naltrexone.

METHOD

Animals

The animals used in these experiments were experimentally naive, adult, male Sprague-Dawley rats that weighed 550–650 grams at the time of the experiments. The rats were housed 2 animals per cage in stainless steel cages (13.5 × 7 × 8 in.) in a restricted access colony room. Animals were given free access to tap water and commercial rat diet (No. 5001; Ralston Purina). Temperature in the colony room was maintained between 22–24°C and the light cycle was 14 hr light:10 hr dark (lights on: 0800 hr). The rats were allowed a 3-week period of acclimation before the experiments were initiated. Each rat was weighed and inspected on a weekly basis to insure optimal health status of the animals.

Cannulation of the Tail Artery and Collection of Blood Samples

The tail artery was used as the cannulation site because the surgical procedures cause minimal trauma to the rats and, in our hands, 7-day postsurgical survival rates of adult rats are 100% with less than 6% loss in body weight relative to presurgery weight. To carry out the cannulation procedure animals were placed in an enclosed chamber and anesthetized with metofane. During surgery, anesthesia was maintained by periodically placing a cone with metofane-impregnated cotton over the nose of the rat. A small incision was made on the ventral side of the tail, the tail artery was exteriorized and the PE-10 section of the catheter (catheters were made by fusing sections of PE-10 and PE-60 tubing) was slowly introduced into the vessel and advanced 8.0 cm to the abdominal aorta. The PE-60 section of the catheter was threaded subcutaneously, and exteriorized at the mid-portion of the animal's back. A steel spring with a steel flat button was positioned over the length of the PE-60 and the button was sutured onto the skin of the rat's back. Catheters were filled with sterile heparin-saline solution (10 U/ml) and plugged into flow through swivels to prevent twisting. The other end of each swivel was connected to an injection pump that maintained a constant flow (5 cc/24 hr) of sterile heparin-saline solution to prevent clotting in the catheters. For blood sampling, the PE-60 section was disconnected from the swivel and blood drawn into a 1.0 ml syringe using a 21 gauge needle (500 μl of blood were drawn for each sample). The blood column remaining in the catheter was flushed with 0.5 ml 1 U/ml heparin-saline solution. Blood samples were transferred to chilled microcentrifuge tubes, centrifuged for 3 min and triplicate plasma aliquots from each sample were transferred into cryotubes maintained at −20°C by immersion in a dry ice bath. Plasma samples were then stored at −80°C until assayed for ACTH, corticosterone and prolactin.

Motion Stressor

The apparatus consisted of a conventional heavy duty reciprocating shaker. The metal platform of the shaker was fitted with a wooden platform divided into six identical compartments. Each of these compartments accommodated snugly a 12″ l × 8″ d × 6″ h plastic rat cage. When the shaker was turned on, the wooden platform with its six plastic cages oscillated from side to side at a rate of 90 excursions per minute. In this way, a smooth and uniform horizontal movement was imparted to all six cages. Rats used in the experiment were placed in the cages on the shaker (1 rat/cage) immediately after surgical implantation of the catheters. Hence, exposure to the motion stressor was accomplished by merely turning on the shaker and required no manipulation of the animals prior to, during, or after application of the motion stimulus. Once the shaker was turned on, the rats were able to remain standing on all fours and typically occupied the center of the cage, avoiding any contact with its sides. This procedure involved no pain or physical discomfort for the rats and was well suited for use with freely moving rats bearing an indwelling tail artery catheter.

Drugs

The tertiary amine compound naltrexone hydrochloride was purchased from Sigma Chemical (St. Louis, MO). The quaternary derivative naltrexone methobromide (compound MRZ 2663) was a generous gift of Boehringer Ingelheim KG. The compounds were dissolved in normal saline to achieve a concentration of the free drug of 2.5 mg/ml. The drugs were administered intraarterially (IA) at a dose of the free drug of 2.5 mg/kg of body weight (injection volume = 1.0 ml/kg body weight). Animals in the vehicle treatment group received an injection of normal saline at a volume of 1.0 ml/kg of body weight.

Radioimmunoassays

Plasma immunoreactive ACTH was measured by the procedure of Nicholson *et al.* (17) in unextracted plasma aliquots. Rabbit anti-ACTH(1–24) serum was purchased from IgG Corporation (Nashville, TN). This antiserum requires position 5–18 of ACTH for full immunoreactivity and shows less than 1% cross-reactivity with human beta-endorphin, beta-MSH, gamma-lipotropin and beta-lipotropin; the serum demonstrates 100% cross-reactivity with ACTH(1–24) and human ACTH(1–39) (20). Tracer (^{125}iodo-ACTH) was purchased from Cambridge Medical Technology (Billerica, MA). Dose-response inhibition of tracer-antibody binding was carried out using human ACTH(1–39) as described previously (3). The intraassay coefficient of variation was 10.2%, and all samples reported in this study were analyzed in a single assay.

Plasma immunoreactive corticosterone was measured in methylene chloride-extracted plasma aliquots using the double antibody procedure described previously (28). Tracer (^{125}iodo-corticosterone) and BSA-conjugated rabbit anticorticosterone were obtained from Cambridge Medical Technology. Standards for the assay were prepared using crystalline corticosterone (Sigma Chemical, St. Louis, MO). The intraassay coefficient of variation was 12.2% and the interassay coefficient of variation was 13.9%.

Plasma immunoreactive prolactin was measured in unextracted

TABLE 1

LEVELS OF PLASMA ACTH, CORTICOSTERONE (CORT) AND PROLACTIN (PROL) MEASURED UNDER RESTING CONDITIONS IN RATS TREATED WITH SALINE (VEH), NALTREXONE HYDROCHLORIDE (NHCl) OR NALTREXONE METHOBROMIDE (NMBr)[a]

		Time in Minutes[b]							
Variable	Drug	0	7.5	15	30	45	60	90	120
ACTH (pg/ml)	VEH	9.4 ± 1.3	5.0 ± 1.1	4.7 ± 1.5	11.1 ± 3.8	9.6 ± 2.3	6.6 ± 2.5	4.9 ± 1.0	4.7 ± 1.1
	NHCl	7.1 ± 1.6	4.9 ± 1.6	6.4 ± 1.3	7.4 ± 1.4	4.1 ± 1.1	8.8 ± 3.4	7.2 ± 2.7	6.1 ± 2.3
	NMBr	7.7 ± 2.5	8.4 ± 3.9	7.1 ± 2.8	7.6 ± 1.8	8.1 ± 2.1	7.9 ± 2.8	5.3 ± 1.6	8.4 ± 3.7
CORT (ng/ml)	VEH	83.1 ± 20.4	42.9 ± 8.3	33.1 ± 5.7	68.4 ± 14.4	90.9 ± 16.2	85.4 ± 15.1	28.6 ± 4.2	37.7 ± 10.6
	NHCl	64.4 ± 14.1	39.1 ± 9.3	64.6 ± 5.2	85.4 ± 17.4	70.0 ± 5.9	89.1 ± 15.9	62.4 ± 19.3	45.0 ± 14.4
	NMBr	55.1 ± 12.2	72.7 ± 19.3	72.7 ± 17.8	80.7 ± 17.4	43.6 ± 10.0	72.6 ± 16.7	43.6 ± 16.2	18.1 ± 3.3
PROL (ng/ml)	VEH	41.0 ± 5.6	42.9 ± 7.0	60.7 ± 4.4	47.7 ± 5.2	42.9 ± 7.1	35.7 ± 7.4	21.4 ± 4.4	23.0 ± 2.6
	NHCl	46.0 ± 6.7	16.9 ± 3.0*	13.6 ± 3.3*	10.3 ± 1.9*	9.6 ± 1.9*	15.4 ± 4.8*	9.9 ± 2.2*	8.4 ± 2.3*
	NMBr	25.6 ± 4.1	21.7 ± 5.2	22.7 ± 4.4*	18.6 ± 4.1*	27.3 ± 6.3	22.9 ± 5.2	25.4 ± 7.4	21.7 ± 4.8

[a]Each value represents the mean ± S.E.M. for n = 7 rats. All compounds were administered as a bolus injection via the intraarterial catheter.

[b]Saline and drug injections were administered 5 min before the time 0 blood sample was drawn.

*Indicates a statistically significant difference by post hoc test ($p<0.05$, Bonferroni procedure) from the corresponding determination in VEH-treated rats.

plasma aliquots using highly specific rabbit anti-rat prolactin antiserum (NIDDK-anti-rPRL-S-9) that was a gift from NIDDK and the National Hormone and Pituitary Program. Tracer for the assay was ^{125}iodo-prolactin (rat) purchased from New England Nuclear. Dose-response inhibition of tracer-antibody binding was carried out using rat prolactin reference preparation (NIDDK-rPRL-RP3) that was also a gift of NIDDK. The intraassay coefficient of variation was 8.7% and all samples reported in the study were assayed in a single run. The second antibody for ACTH, corticosterone and prolactin assays was goat anti-rabbit gamma-globulin (Antibodies Inc., Davis, CA). For all assays, precipitation of antigen-antibody complexes was accomplished by addition of 20% polyethylene glycol (Carbowax 8000, Fisher Scientific) in assay buffer.

Experimental Design

The experiments were designed to determine whether blockade of central and/or peripheral opiate receptors would alter ACTH, corticosterone and prolactin responses to the motion stressor. Rats were anesthesized and tail artery catheters were implanted into each rat as already described. Following surgery rats were housed individually in the plastic cages mounted on the motion apparatus. All animals were allowed 2 days of undisturbed postsurgical recovery prior to the experiments. On postoperative day 3, rats were randomly assigned to receive one of three possible drug treatments, i.e., vehicle (VEH), naltrexone hydrochloride (NHCl) or naltrexone methobromide (NMBr). At 5 min after drug administration a blood sample was drawn from each rat (this was the time 0 sample). Thereafter, 50% of the rats in each of the 3 drug treatment groups were exposed to a 30-min session of the motion stressor. Blood samples were drawn from each of these rats at 7.5, 15 and 30 min during motion stress and at 15, 30, 60 and 90 min after cessation of the stress session. The remaining 50% of the rats in each drug treatment group were not exposed to the stressor, but blood samples were drawn from these rats at times comparable to those of their stressed counterparts. On postoperative day 4 all rats were allowed to rest undisturbed. On postoperative day 5 the experiment was repeated in a manner identical to day 3, except that rats exposed to drug and stress on day 3 received only drug on day 5, and rats exposed to drug only on day 3 received drug and stress drug on day 5. In this manner, blood samples were obtained from rats in each drug treatment group under resting as well as stressed conditions.

Data Analysis

The results were analyzed by one-way repeated measures analysis of variance (ANOVA). Comparisons between individual means by post hoc test were done using the Bonferroni procedure, and the criterion for statistical significance was set at $p<0.05$. In order to determine treatment effects on the poststress decline of ACTH and corticosterone, the rate of disappearance (K_d) from the circulation of each of these hormones was calculated according to

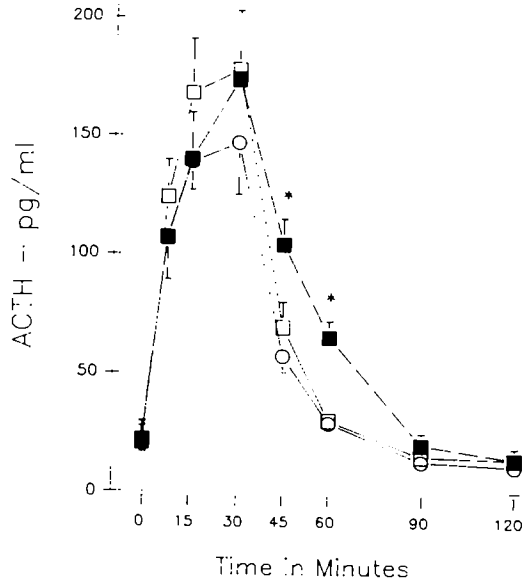

FIG. 1. Stress-induced levels of plasma ACTH measured in rats pretreated with saline (open circle, continuous line), naltrexone hydrochloride (closed square, broken line) or naltrexone methobromide (open square, continuous line). Each value represents the mean ± S.E.M. for n = 7 rats. Where not shown, the S.E.M. was contained within the symbol. Naltrexone hydrochloride and naltrexone methobromide were administered at a dose of 2.5 mg/kg of body weight (doses calculated as the free drug) 5 min before the time 0 blood sample was drawn. The stressor was applied beginning at time 0 and ending at 30 min. The asterisk indicates a significant post hoc difference ($p<0.05$, Bonferroni procedure) from the corresponding determination in vehicle-treated rats.

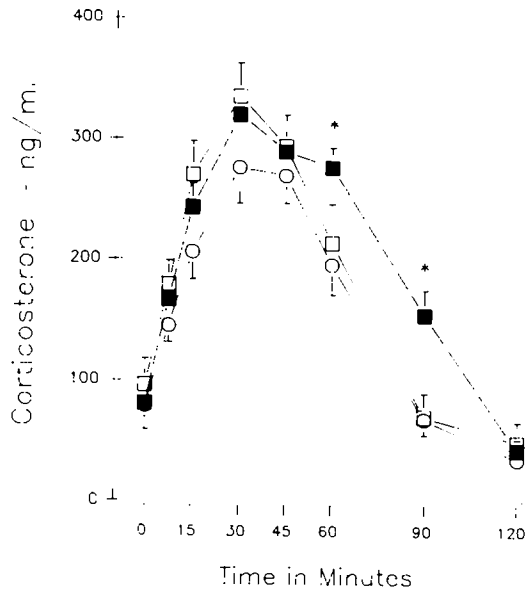

FIG. 2. Stress-induced levels of plasma corticosterone measured in rats pretreated with saline (open circle, continuous line), naltrexone hydrochloride (closed square, broken line) or naltrexone methobromide (open square, continuous line). Each value represents the mean ± S.E.M. for n = 7 rats. Where not shown, the S.E.M. was contained within the symbol. All other explanations are as in the legend to Fig. 1.

the formula (14): $K_d = 2.303 (\log H_1 - \log H_2)/T_2 - T_1 = \% \text{ min}^{-1}$, where H_1 and H_2 were the hormone levels measured at times (T) 1 and 2, respectively.

RESULTS

Table 1 presents the hormone levels that were measured under resting conditions in saline- and drug-treated rats. Levels of ACTH were unaffected by either of the drugs. Statistically, this was confirmed by the absence of a significant main effect of drug, $F(2,18) = 0.12$, $p = 0.88$. Likewise, the lack of a significant effect of time, $F(7,126) = 1.01$, $p = 0.42$, demonstrated that the sampling

TABLE 2

RATE OF DECLINE (K_d) OF ACTH AND CORTICOSTERONE (CORT) MEASURED AFTER CESSATION OF EXPOSURE TO THE MOTION STRESSOR IN RATS TREATED WITH SALINE (VEH), NALTREXONE HYDROCHLORIDE (NHCl) OR NALTREXONE METHOBROMIDE (NMBr)[a]

	K_d (% min $^{-1}$)[b]		
Variable	VEH	NHCl	NMBr
ACTH (30 to 60 min)	5.3 ±0.42	3.3 ±0.30*	5.9 ±0.57
CORT (30 to 90 min)	2.5 ±0.26	1.3 ±0.15*	2.8 ±0.30

[a]Each value represents the Mean ± S.E.M. for n = 7 rats.

[b]Values for ACTH are given in pg/ml; values for CORT are given in ng/ml.

*Indicates a statistically significant difference by post hoc test ($p<0.05$, Bonferroni procedure) from the corresponding K_d value of VEH-treated rats.

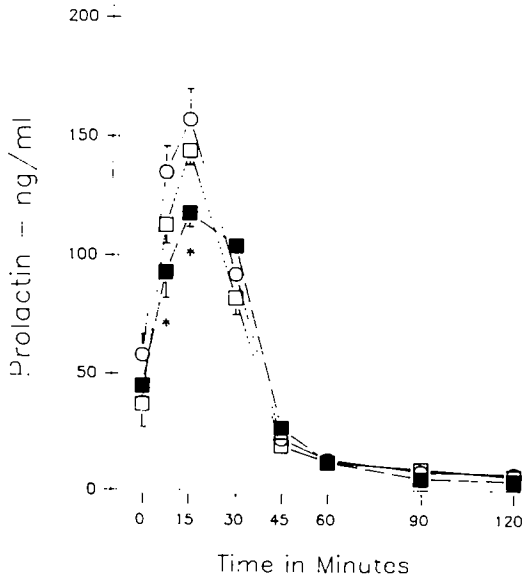

FIG. 3. Stress-induced levels of plasma prolactin measured in rats pretreated with saline (open circle, continuous line), naltrexone hydrochloride (closed square, broken line) or naltrexone methobromide (open square, continuous line). Each value represents the mean ± S.E.M. for n = 7 rats. Where not shown, the S.E.M. was contained within the symbol. All other explanations are as in the legend to Fig. 1.

procedure itself did not influence ACTH levels. Resting levels of corticosterone showed slight fluctuations across treatment groups, as well as during the sampling procedure. The overall main effect of drug treatment was statistically not significant, $F(2,18) = 0.32$, $p = 0.73$, but the effect of time on corticosterone levels was significant, $F(7,126) = 5.10$, $p = 0.001$. However, the temporal fluctuation of this response appeared to be nonspecific and within the range of expected resting levels of the hormone. Thus, it is unlikely that this response variation reflected a sampling-induced stress response. Resting levels of prolactin showed a significant main effect of drug, $F(2,18) = 14.85$, $p = 0.001$. This effect was most pronounced in NHCl-treated rats as demonstrated by the substantial and sustained drop in levels of this hormone that was seen by the 7.5 min determination (i.e., 12.5 min postdrug administration). In the case of NMBr-treated rats, initial (i.e., time 0) prolactin levels were somewhat lower than those of the VEH group but remained fairly constant throughout the sampling procedure. As determined by post hoc test, prolactin levels in the NHCl-treated group of rats were significantly lower than levels in the vehicle group from the 7.5-min determination until the end of the experiment.

The motion stress-induced ACTH responses measured in vehicle- and in drug-treated rats are presented in Fig. 1. For each of the three groups of rats, ACTH levels rose continuously throughout the stress session and reached a peak at 30 min of stress exposure. A trend was observed for ACTH concentrations to increase to a higher level in NHCl- and NMBr-treated rats compared to saline controls, but this was not statistically significant as determined by post hoc test. By 30 min into the poststress interval (i.e., time = 60 min), ACTH levels had returned to prestress values in control and in NMBr-treated rats, but remained substantially elevated in NHCl-treated animals. Thus, it appeared that the main effect of NHCl was to prolong the stress-induced ACTH response. Given that this effect was seen only at the 45 and 60 min time points, it is not surprising that, as determined by repeated measures ANOVA of the results, neither the main effect of drug, $F(2,18) = 1.02$, $p = 0.38$, nor the drug × time interaction, $F(14,126) = 1.38$, $p = 0.17$, was statistically significant. However, the data presented in Table 2 demonstrated a pronounced effect of naltrexone hydrochloride on the rate of decline (K_d) of ACTH during the 30- to 60-min interval of the experiment. Analysis of the K_d results by one-way ANOVA indicated that the main effect of drug treatment was statistically significant, $F(2,18) = 9.63$, $p < 0.001$. Likewise, analysis of the K_d data by post hoc test confirmed that the rate of decline of ACTH was significantly slower in NHCl- than in vehicle-treated rats, but was comparable between NMBr-treated and control animals. Hence, the data showed that blockade of central but not of peripheral opiate receptors significantly prolonged the motion stress-induced ACTH response.

Stress-induced levels of corticosterone that were measured in control and drug-treated animals are presented in Fig. 2. The corticosterone responses resembled and were consistent with the ACTH responses that were presented in Fig. 1. The highest hormone values were seen at 30 min into the stress session, and no statistically significant differences in the peak level of corticosterone were seen between any of the three treatment groups. During the poststress period vehicle- and NMBr-treated rats showed normal levels of corticosterone by 60 min (i.e., time = 90 min) after the end of the stress session. By contrast, in NHCl-treated rats, levels of the hormone were still significantly elevated at that time. Thus, in good agreement with the ACTH results, the data in Fig. 2 showed that pretreatment with the centrally but not with the peripherally acting form of naltrexone, prolonged the stress-induced corticosterone response. Statistically, as determined by repeated measures ANOVA, the main drug effect was not statistically significant, $F(2,18) = 2.10$, $p = 0.15$, and neither was the time × drug interaction, $F(2,18) = 1.71$, $p < 0.06$. However, the effect of NHCl on the rate of decline of corticosterone (shown in Table 2) was highly significant as determined by one-way ANOVA, $F(2,18) = 8.86$, $p = 0.002$. Analysis of the data in Table 2 by post hoc test also showed that the K_d values of NHCl-, but not NMBr-treated rats differed significantly from control values.

The prolactin results are summarized in Fig. 3. As can be seen, the stress-induced increase in circulatory levels of prolactin was comparable between rats treated with saline and animals treated with naltrexone methobromide. By contrast, in rats pretreated with naltrexone hydrochloride the peak level of the stress-induced prolactin response was significantly lower than that seen in the vehicle-treated control group. Statistically, the main effect of drug was not significant, $F(2,18) = 2.43$, $p = 0.12$, but the drug × time interactive term was significant, $F(14,126) = 3.09$, $p = 0.001$. Likewise, comparisons of individual means by post hoc test showed that at 7.5 and 15 min prolactin levels were significantly lower in NHCl-treated rats compared to vehicle controls. For each of the 3 rat groups, prolactin levels declined progressively after the 15 min time point to a level that was still below the prestress value by 120 min. This decline in prolactin levels to values below the level measured before stress exposure was not modified by any of the drug treatments. Thus, the data showed that pretreatment with the tertiary but not the quaternary naltrexone derivative attenuated the stress-induced increase in prolactin levels, and that the poststress suppression of this hormone was not affected by any of the compounds administered.

DISCUSSION

The results of these experiments demonstrated that pharmacological antagonism of opiate receptor function prolonged stress-induced HPA axis responses. The data further showed that this effect was dependent on opiate-mediated effects within the central nervous system (CNS), since blockade of peripheral opiate receptors had no effect on these responses. It should be emphasized that the present results were obtained using a motion stressor which minimized nociceptive or tactile stimulation of the animals. Reports in the literature have shown that administration of opiate receptor antagonists can lower the threshold for pain perception (10) and that opiate receptor-mediated events such as certain forms of stress-induced analgesia (29) can be localized within specific regions of the body (35). Hence, effects of opiate receptor antagonists on responses to stressors, like electric shock, which involve direct physical stimulation of the animals could result, in part, from blockade of stress-induced analgesia and/or from localized or generalized hyperalgesia. These effects, in turn, could alter the perceived aversiveness of the stressor and thus modify HPA axis responses indirectly, in a manner that would be difficult to distinguish from a direct opiate action on this system. By contrast, it is unlikely that HPA axis responses to the motion stimulus used in this study could be significantly affected by drug-induced hyperalgesic or antianalgesic effects, because nociceptive and/or tactile perception probably play only a minor role in responses to this stressor. Thus, it seems warranted to conclude that the drug-associated effects observed in this study reflect a direct, opiate-mediated modulatory influence on stress-induced HPA axis function.

The dose of naltrexone used in these experiments is sufficient to antagonize opiate receptor function, since similar doses of the shorter acting antagonist naloxone are known to reverse morphine-induced analgesia (12). At this dose level we observed no significant effect of either the tertiary or quaternary naltrexone derivatives on resting levels of ACTH and corticosterone. This observation is in good agreement with reports by other investigators which showed that low doses of the opiate blocker did not

alter resting levels of these hormones (8, 9, 31). Because there was no effect of NHCl on basal levels of ACTH and corticosterone, it can be concluded that the drug affected stress-induced HPA axis function. This agrees with other reports which showed that endocrine (31), electrophysiological (1) and neurochemical (30) effects of opiate receptor antagonists that were evident in rats exposed to stress were not seen in resting control animals. In addition, our results confirmed earlier work by Tapp *et al.* (31) who found that pharmacological blockade of opiate receptors did not alter the peak level of the stress-induced corticosterone response, but delayed its decline during the poststress interval. The data in Table 2 showed that this effect was independent of stress-induced corticosterone levels, since the rate of decline (K_d) of the response was significantly faster in NMBr-compared to NHCl-treated animals, whereas 30-min corticosterone levels were virtually the same in these two groups of rats. Furthermore, the present data indicated that the prolongation of the stress-induced corticosterone response may be due to an effect on ACTH since a prolongation of this response (Fig. 1) preceded the delay in the decline of corticosterone levels (Fig. 2) that was seen in NHCl-compared to NMBr- or saline-treated rats.

The quaternary naltrexone derivative was used to determine whether opiate effects on stress-induced HPA axis responses involved central and/or peripheral opiate-mediated actions. The peripheral-only action of quaternary derivatives of opiate receptor blockers has been established by previous studies using radiolabeled analogues which have shown minimal penetration of these compounds into the CNS (27) and by inability of systemically (5,12) but not intracerebrally administered (5) quaternary opiate antagonists to block morphine-induced analgesia. Peripheral effects of opiate blockers on stress-induced HPA axis responses can be inferred from studies which have shown that endogenous opioid peptides decrease the response of adrenocortical cells to ACTH-stimulated corticosterone release (13,22). However, in the present experiments, we found that administration of the quaternary naltrexone derivative did not alter the magnitude or the time course of the stress-induced ACTH and corticosterone responses, relative to saline-treated rats. Previously (25), it was reported that opiate receptor antagonist effects on HPA axis responses were not abolished by complete hypothalamic deafferentation. Hence, the present observation that naltrexone methobromide did not influence HPA axis responses to stress, coupled with the previous report (25), strongly implicate the hypothalamus as the anatomical locus at which opiate receptor antagonists act to enhance or prolong stress-induced ACTH responses. We would therefore speculate that opioid peptides may be important mediators of the termination of the stress-induced corticotropin releasing hormone (CRH) secretory response, and that centrally acting opiate antagonists delay the decline of stress-induced HPA axis responses by prolonging the release of CRH.

Our results are also in good agreement with the work of other investigators (24, 26, 33) who showed that administration of opiate receptor antagonists diminished both resting and stress-induced prolactin levels. In addition, we found that the quaternary naltrexone derivative was inactive with regard to attenuating prolactin responses. Earlier reports showed that opiates did not affect prolactin responses at the pituitary level (23) and that naloxone retained its ability to inhibit prolactin release in the complete hypothalamic deafferented rat preparation (26). Hence, the present experiments provide further evidence that the actions of opiate receptor antagonists to inhibit prolactin release are mediated within the CNS, probably at the hypothalamic level.

In summary, these experiments provided strong evidence to indicate that blockade of opiate receptor-mediated mechanisms within the CNS prolonged stress-induced ACTH and corticosterone responses. Furthermore, our results, coupled with findings by other investigators (25), suggest that the effect of opiate antagonists may be mediated at the hypothalamic level. We further speculate that a possible role of hypothalamic opiate mechanisms may be to terminate release of CRH after exposure to a stressful situation has ended.

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