Pharmacology Biochemistry and Behavior, Vol. 53, No. 4, pp. 927–933, 1996

0091-3057/96 $15.00 + .00

SSDI 0091-3057(95)02139-6

Effects of β-FNA on Sympathoadrenal, Cardiovascular, and Analgesic Responses to DAMPGO at Rest and During Stress

ABDULGHANI A. HOUDI,[1] LESLEY MARSON,[2] KATHERINE E. DAVENPORT AND GLEN R. VAN LOON[3]

Division of Endocrinology and Metabolism, Department of Medicine, University of Kentucky and VA Medical Center, Lexington, KY

Received 26 March 1995; Revised 12 August 1995; Accepted 25 August 1995

HOUDI, A. A., L. MARSON, K. E. DAVENPORT AND G. R. VAN LOON. *Effects of β-FNA on sympathoadrenal, cardiovascular, and analgesic responses to DAMPGO at rest and during stress.* PHARMACOL BIOCHEM BEHAV 53(4) 927–933, 1996. – To elucidate further the role of mu-opioid receptors in mediating analgesia and cardiovascular function at rest and during stress, rats were pretreated ICV with either saline (5 μl) or β-funaltrexamine (β-FNA, 5 nmol/5 μl), a noncompetitive opioid receptor antagonist that inactivates irreversibly mu receptors, 2 days prior to [D-Ala^2,N MePhe^4,Gly^5-ol]enkephalin (DAMPGO, 1 nmol, ICV) administration. Pretreatment with β-FNA blocked DAMPGO-induced analgesia as measured by the tail-flick test. DAMPGO also produced an increase in blood pressure (BP), sympathoadrenal outflow, and a bradycardia. Pretreatment with β-FNA converted the DAMPGO-induced bradycardia to a tachycardia, significantly reduced the DAMPGO-induced increase in epinephrine by 60%, and the norepinephrine response by 45%, and attenuated mildly the increase in BP due to DAMPGO. In saline-treated rats, restraint stress evoked an increase in HR, BP, and plasma catecholamines. Pretreatment with β-FNA partially attenuated the increase in HR in response to stress. In the presence of DAMPGO, restraint stress resulted in a further bradycardia, which was significantly blocked by pretreatment with β-FNA. Stress also produced increases in BP and plasma catecholamines, which were not prevented by pretreating rats with β-FNA. These results indicate that β-FNA may not have inactivated all the receptors accessible to DAMPGO which control BP, or alternatively, β-FNA may selectively inactivate a subtype of mu receptors. In addition, brain mu opioid receptors appear to be significantly involved in mediating supraspinal analgesia and regulating parasympathetic outflow to the heart and sympathoadrenal release of catecholamines.

Mu-opioid receptors β-FNA DAMPGO Analgesia Cardiovascular responses Restraint stress Plasma catecholamine

BRAIN OPIOIDS have been implicated in the regulation of autonomic function at rest and during stress (1,11,12,16,31). Central administration of agonists that interact competitively with the mu receptor, such as morphine and DAMPGO, produce dose-dependent, naloxone-reversible changes in cardiovascular responses (1,7,9,11,12,16,22) and increase the nociceptive threshold (5,21,22). Stress induces analgesia and potentiates the analgesic effects of opioids such as morphine and DAMPGO (2,3,15). Interaction among opioid peptides and stress in the regulation of cardiovascular function has been reported (11,16). During stress, mu-opioid receptor stimulation facilitates sympathetic and parasympathetic outflow (11,16). However, there still remains considerable uncertainty over the precise role of brain opioids in modulating cardiovascular functions. In addition, subtypes of mu opioid receptors have been proposed (19,23,26,37,39). However, it is not clear which of the proposed subtypes of mu receptors are important in such action.

[1] Requests for reprints should be addressed to A. A. Houdi at his present address: College of Pharmacy and Tobacco and Health Research Institute, University of Kentucky, Rose Street, Lexington, KY 40536.
[2] Present address: Division of Urology, Department of Surgery, 427 Burnett-Womack, University of North Carolina, Chapel Hill, NC 27599.
[3] Present address: Stilley Professional Building, George McClain Drive, Benton, KY 42025.

The effects of competitive agonists such as morphine and DAMPGO usually are proportional to receptor occupancy. β-Funaltrexamine (β-FNA) is a noncompetitive opioid antagonist that binds covalently and inactivates irreversibly mu-opioid receptors (29,34). Previous studies have shown that central administration of β-FNA will antagonize mu-preferring agonists more effectively than delta or kappa agonists (30,35). Thus, the potential effect of endogenous and exogenously administered mu-opioid agonists would be minimized after pretreatment with β-FNA. We sought in the present study to determine whether, consistent with the hypothesis generated in the tolerance model, downregulation of the brain mu receptor population would have different effects on the central cardiovascular and antinociceptive actions of the mu-selective agonist DAMPGO. The present study also examines further the contribution of brain mu-opioid receptors in modulating stress-induced changes in blood pressure, heart rate, and plasma catecholamines in rats pretreated centrally with either saline or β-FNA. Preliminary data have been presented in abstract form (10).

METHODS

General Procedures

Male Sprague-Dawley rats (275-350 g, Harlan Sprague-Dawley Inc., Indianapolis, IN) were housed individually in an environmental room at 24°C with controlled light-dark cycles (lights on 0700-1900 h). Food and water were administered ad lib. Animals were prepared surgically for experimentation as follows: under equithesin anesthesia (3.0 ml/kg), a polyethylene catheter (PE-50) prefilled with heparinized saline (50 units/ml) was placed into the left internal carotid artery, and a stainless steel guide cannula (Plastic Products, Roanoke, VA) was implanted 1.0 mm above the left lateral cerebral ventricle (coordinates: 0.8 mm caudal to bream, 1.5 mm lateral, 3.2 mm below the skull). The guide cannula was secured to the skull with dental acrylic and screws. A 28 gauge dummy cannula remained in the implanted cannula except during intracerebroventricular injections. The arterial catheter was passed out of the home cage through a stainless steel spring attached to the animal's back. This arrangement permitted blood sampling and cardiovascular monitoring in the conscious, freely moving rat. Rats were allowed to recover from surgery for 3 days before use in an experiment.

Experimental Protocol

Forty-eight hours before an experiment, rats received an ICV injection of either β-FNA (5 nmol/rat) or saline in a volume of 5 μl over 2-min period using a programmable syringe pump (Tracer Atlas, Houston, TX) and 50 μl syringe attached to the injector by PE-10 tubing. The rats remained in their home cages for the injections. They were removed only momentarily to connect and disconnect the ICV injector.

On the day of the experiment, 30 min before drug administration, animals were handled briefly to lower a drug-filled injector through the guide cannula into the lateral ventricle. The rats were then returned to the home cages where they were allowed to recover from the stress of handling. Thirty minutes later, microinjection of DAMPGO (1 nmol/5 μl/rat) or saline (5 μl/rat) made over a 2-min period using a programmed syringe pump. The four treatment groups will be described as saline/saline; β-FNA/saline; saline/DAMPGO, and β-FNA/DAMPGO.

The effects of DAMPGO or saline on plasma catecholamine and cardiovascular responses to restraint stress were assessed 20 min after drug administration, when the response to DAMPGO had plateaued. Rats were removed from their cage and placed into a rigid, semicircular Plexiglas rodent restrainer, modified with a slot to allow free movement of the cannula housing. The tail gate was adjusted to prevent any significant movement by the animal without impairing circulation.

Heart rate (HR) and systolic, diastolic, and mean arterial blood pressure were recorded continuously throughout the experimental period. These analog signals were digitized by an analog-to-digital (A/D) converter (Buxco Electronics, Sharon, CT), averaged over 6-s epochs, and stored using a Buxco Data Logger and an IBM PC/AT.

Blood samples (0.5 ml) for determination of plasma catecholamine concentrations were collected from the carotid artery at 5 min before and 20 min after injection of DAMPGO and 5 min after the onset of restraint stress. Each sample was replaced by an equal volume of saline. Epinephrine (EPI) and norepinephrine (NE) were measured in plasma (50 μl, in duplicate) using a radioenzymatic assay procedure (27).

At the conclusion of the experiment, each ICV injection site was verified by gross dissection of the rat brain after a 5 μl injection of fast green dye in deeply anesthetized animals.

Analgesia Testing

Rats were acclimated to the tail-flick test as described by D'Amour and Smith (4), with modification by Gellert and Holtzman (8), two times before the actual experiment was run. The analgesic effect of the mu opioid receptor agonist, DAMPGO, was assessed 28 min after ICV administration of DAMPGO, and continued at 3 min intervals for 12 trials. An 8-s cutoff time was used to prevent damage to the tail. At the conclusion of the experiment, each ICV injection site was verified by gross dissection of the rat brain after a 5 μl injection of fast green dye in deeply anesthetized animals.

Measurements of analgesia were expressed as:

$$\%\ \text{Maximum Possible Response (\% MPR)} = \frac{\text{post drug latency} - \text{baseline latency}}{\text{time (8.0 s)} - \text{baseline latency}} \times 100\ \text{cutoff}$$

Data Analysis

The levels of blood pressure and heart rate for each rat were obtained as 2 min averages over the whole course of the experiment. The data are presented as mean ± SE for the given number of rats. Data were analyzed using Asyst Scientific Software (Macmillan Software Company, NY). Statistical analyses were analysis of variance (ANOVA) for repeated measures, followed by Duncan's new multiple-range test or unpaired *t*-test, as appropriate.

Drugs

Drugs used in these experiments were: equithesin, prepared by mixing chloral hydrate (2.13 g, Sigma Chemical Co., St. Louis, MO), magnesium sulfate (1.07 g), propylene glycol (14.1 ml, Fisher Scientific Co., Fairlawn, NJ), ethanol 3.8 ml), water (24.6 ml), and sodium pentobarbital 64.8 mg/ml (7.5 ml, Butler, Columbus, OH); β-FNA (RBI, Natick, MA); and DAMPGO (Peninsula Laboratories, San Carlos, CA).

RESULTS

Baseline Values (Table 1)

Pretreatment with β-FNA did not affect basal levels of blood pressure, heart rate or plasma catecholamines (Table 1). There were no significant differences noted among experimental groups for any parameters.

Effects of Mu Opioid Receptor Inactivation by β-FNA on Cardiovascular and Sympathoadrenal Responses to DAMPGO

To examine the effect of irreversible inactivation of brain mu-opioid receptors on the autonomic changes produced by DAMPGO, rats were pretreated with β-FNA or saline administered ICV. Two days later, both groups of rats received DAMPGO. Microinjection of DAMPGO significantly increased systolic, diastolic, and mean blood pressures (Fig. 1). The peak increase in blood pressure occurred at 12 min, then the response plateaued. Pretreatment with β-FNA attenuated the DAMPGO-induced increase in blood pressure (Fig. 1). The initial rate of increase was similar in both groups; however, the β-FNA group plateaued at a lower level that the saline group. Microinjection of DAMPGO evoked a small but significant bradycardia in control animals; this was converted to a tachycardia in the β-FNA treated group (Fig. 2). The response to DAMPGO was significantly different in the β-FNA group compared to the saline group (ANOVA, repeated measures over time, $p < 0.001$).

In control groups (saline/saline, $n = 5$ and β-FNA/saline, $n = 5$), ICV administration of saline in place of DAMPGO did not produce appreciable changes in cardiovascular responses (data not shown). Furthermore, there were no differences in cardiovascular responses between rats pretreated 48 h earlier with saline (saline/saline) or β-FNA (β-FNA/saline).

Microinjection of DAMPGO produced significant increases in plasma EPI and NE concentrations. Plasma EPI increased by 15.43 ± 6.56 nM and plasma NE increased by 2.51 ± 0.77 nM 20 min after administration of DAMPGO. Pretreatment with β-FNA significantly reduced the DAMPGO-induced increase in EPI by ~60% and NE response by ~45% (Fig. 3). Mincroinjection of saline in place of DAMPGO had no effect on plasma epinephrine and norepinephrine in both groups of rats pretreated 48 h earlier with either saline (saline/saline) or β-FNA (β-FNA/saline) (data not shown).

Effects of β-FNA on Stress-Induced Changes in Cardiovascular and Sympathoadrenal Responses in the Presence of DAMPGO

The effect of restraint stress was tested 20 min after ICV administration of DAMPGO, when the cardiovascular responses to DAMPGO had plateaued. Restraint stress resulted in small further increases in systolic, diastolic, and mean blood pressure in both the saline and β-FNA pretreated group. There were no significant differences between the saline and

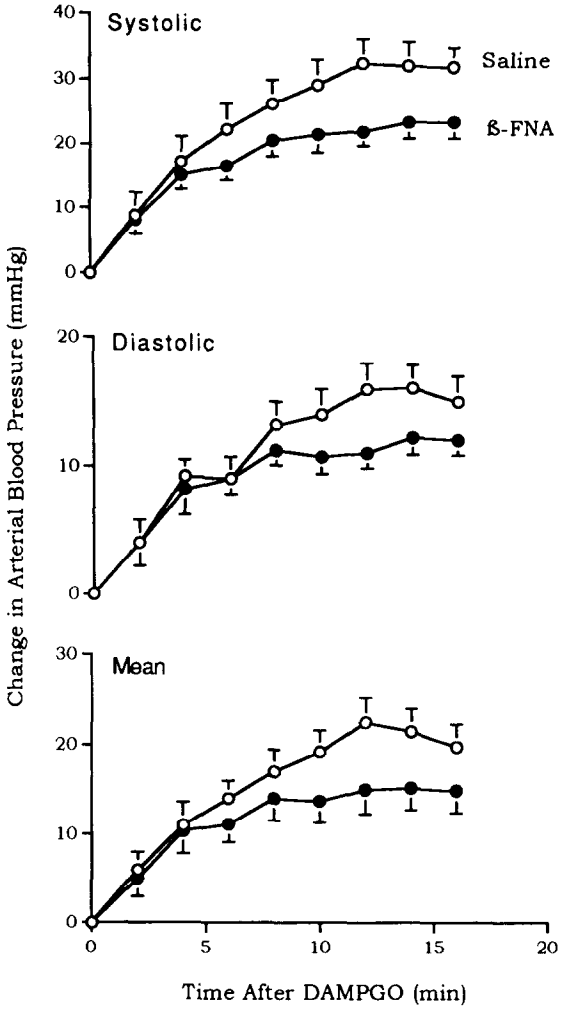

FIG. 1. Effect of DAMPGO on blood pressure in rats pretreated centrally 48 h earlier with either saline or β-FNA. Groups of rats received a microinjection of DAMPGO (1 nmol ICV) at time = 0. The mean ± SE changes in systolic, diastolic, and mean blood pressures were obtained as a 2-min average from basal levels (pre-DAMPGO recordings).

TABLE 1

BASELINE VALUES FOR CARDIOVASCULAR PARAMETERS AND PLASMA CATECHOLAMINES

Treatment Groups	Heart Rate (beats/min)	Blood Pressure (mmHg)			Plasma Catecholamines (nM)	
		Systolic	Diastolic	Mean	Epinephrine	Norepinephrine
Saline ($n = 13$)	401 ± 12.3	113 ± 3.3	82 ± 2.7	92 ± 2.5	0.92 ± 0.6	1.1 ± 0.45
β-Funaltrexamine ($n = 14$)	392 ± 9.3	113 ± 3.4	84 ± 3.2	94 ± 2.9	0.99 ± 0.6	1.23 ± 0.7

Data represent mean ± SEM of values obtained 2 days after ICV administration of saline or β-funaltrexamine.The value for each cardiovascular parameter used from each rat represents the mean for 10 data points taken during the 2 min prior to treatment with DAMGO.

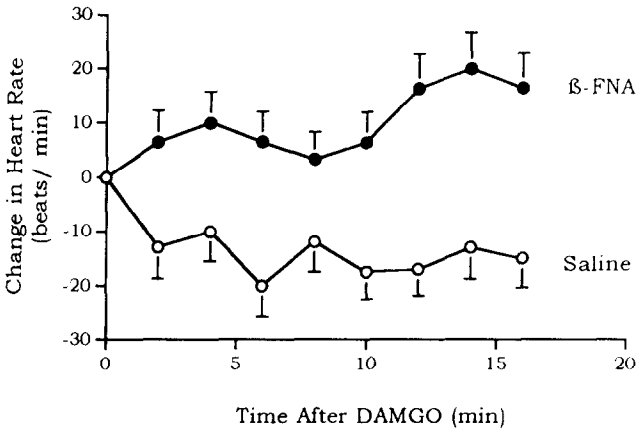

FIG. 2. Effect of DAMPGO on heart rate in rats pretreated centrally 48 h earlier with either saline or β-FNA. Groups of rats received a microinjection of DAMPGO (1 nmol ICV) at time = 0. The mean ± SE changes in heart rate were obtained as a 2-min average from basal levels (pre-DAMPGO recordings).

β-FNA groups (Fig. 4). Restraint stress produced a large fall in heart rate in the saline-pretreated group. This bradycardia was substantially greater than that seen in response to DAMPGO (1 nmol). In the β-FNA-pretreated group, restraint stress produced only a small bradycardia (Fig. 5). This bradycardia was significantly different from the response in the saline-pretreated group ($p < 0.001$) and brought the heart rate back to basal levels. Restraint stress, in the presence of exogenously administered DAMPGO, produced an increase in plasma catecholamines. β-FNA did not alter the plasma catecholamine response to restraint stress (Fig. 3).

Restraint stress in saline-treated rats (the saline/saline group), in place of DAMPGO, in the absence of β-FNA, evoked a significant increase in HR (Δ 120 ± 16 beats/min, Fig. 5) and in plasma EPI (Δ 3.2 ± 0.52 nM) and NE (Δ 1.8 ± 0.3 nM) with a mild increase in BP (Fig. 4). Pretreatment with β-FNA (β-FNA/saline group) 48 h earlier attenuated this increase in HR (+79 ± 13 beats/min, but did not reach statistical significance) due to stress in rats receiving ICV saline in place of DAMPGO (Fig. 5). Pretreatment with β-FNA also had no significant effect on BP or plasma catecholamines in response to stress compared to the saline pretreated group.

Effect of β-FNA on Analgesia Produced by DAMPGO

ICV administration of DAMPGO (1 nmol) produced 100% analgesia in the saline-treated group; maximal analgesia was present 30 min after injection of DAMPGO and was maintained for a further 30 min (Fig. 6). Pretreatment with β-FNA 48 h prior to DAMPGO blocked markedly the analgesic effect of DAMPGO (Fig. 6).

DISCUSSION

The dose of β-FNA used in the present study antagonized the analgesic effects of DAMPGO 48 h later. These results agree with previous reports that central administration of β-FNA antagonizes the analgesic effects of morphine for up to 5 days (24,34) and is consistent with a decrease in mu-opioid receptors. After 48 h, we presume that the remaining β-FNA is effectively coupled to the receptors and that the amount of free drug is near zero. β-FNA did not completely prevent DAMPGO analgesia (~80% inhibition). Other opioid receptors (13,38), in addition to mu-receptors or possibly other subtypes of mu-receptors, mediate a portion of DAMPGO analgesia, and these would not be affected by β-FNA. Intrathecal administration of β-FNA also antagonized the analgesic effect of both morphine and DAMPGO administered intrathecally (18). We have previously demonstrated (5) that ICV administration of β-FNA blocked the analgesic effect of SC morphine in rats, which further supports the thesis that supraspinal sites containing mu opioid receptors contribute to opioid-induced analgesia as assessed by the tail-flick test.

Pretreatment with β-FNA blocked the DAMPGO-induced fall in HR and increase in sympathoadrenal outflow. Additionally, the profound bradycardia seen upon restraint stress in rats pretreated with DAMPGO was blocked by pretreatment with β-FNA. Brain opioid receptors have been shown to be involved in the regulation of cardiovascular and respiratory functions (1,6,11,12,16). ICV administered DAMPGO or the delta agonist [D-Pen^2,D-Pen^5]enkephalin (DPDPE), produced dose-related increases in blood pressure and sympathoadrenal outflow. These effects were blocked by a mu-selective dose of

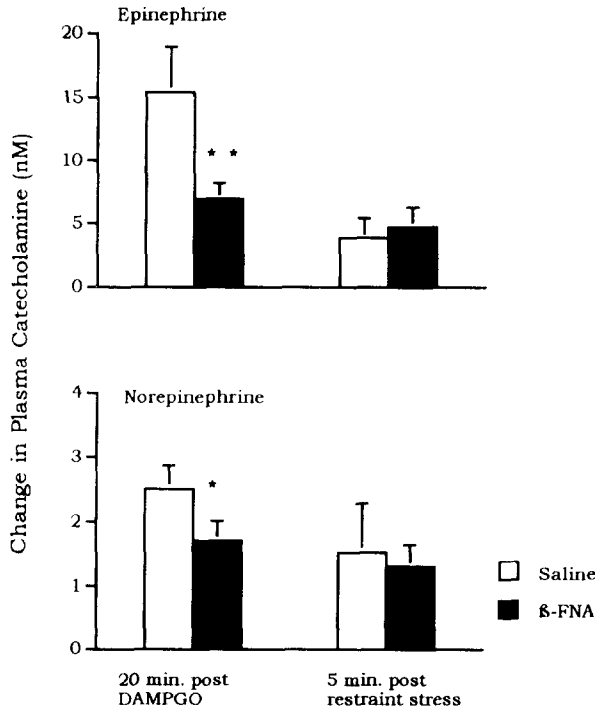

FIG. 3. Effect of DAMPGO and restraint stress on plasma epinephrine and norepinephrine in rats pretreated centrally 48 h earlier with either saline or β-FNA. DAMPGO was administered at time = 0 and restraint stress was imposed 20 min later. Data (mean ± SE for eight to nine rats per group) are presented as the average change in plasma epinephrine and norepinephrine concentration before and after DAMPGO treatment and before and after stress. Basal epinephrine = 0.92 ± 0.6 nM and 0.99 ± 0.6 nM for saline and β-FNA pretreated groups, respectively; basal norepinephrine = 1.1 ± 0.45 nM and 1.23 ± 0.7 nM for saline and β-FNA pretreated groups, respectively. Significant decrease in the plasma catecholamine response to β-FNA pretreated rats compared to saline pretreated rats is represented by $*p < 0.05$, $**p < 0.01$.

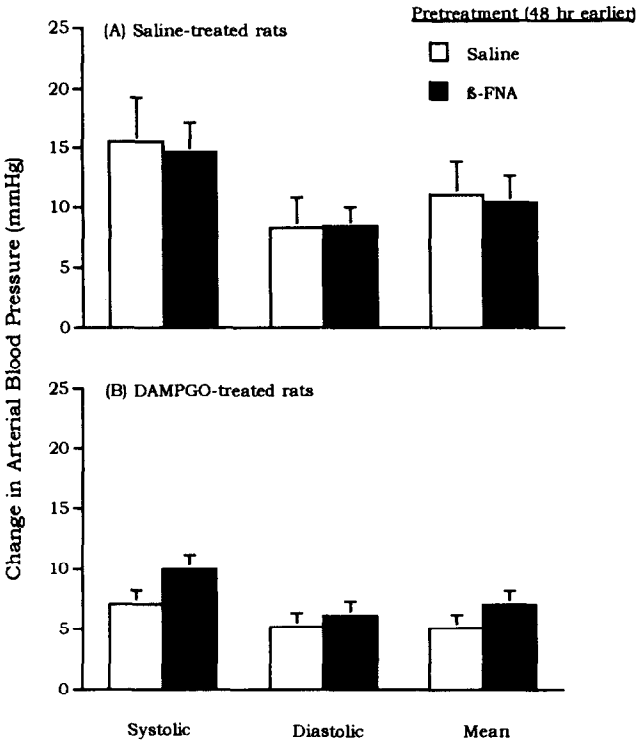

FIG. 4. Effect of restraint stress on systolic, diastolic and mean arterial blood pressure in the presence of (A) saline or (B) DAMPGO. Rats were pretreated centrally 48 h earlier with either saline or β-FNA. Saline or DAMPGO was injected ICV 21 min before the onset of stress. Peak blood pressure responses are shown at 1-2 min after the onset of restraint compared to the level immediately before restraint.

naloxone (0.4 mg/kg) but not by the delta receptor antagonist, ICI 174864, indicating the involvement of brain mu opioid receptors (12,16). In this study, pretreatment with β-FNA blocked the DAMPGO-induced bradycardia, providing further evidence that the increase in parasympathetic outflow to

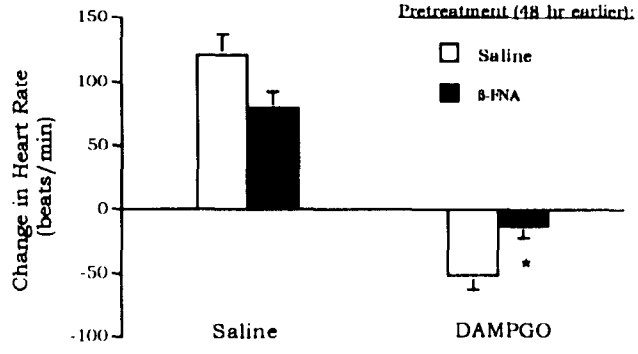

FIG. 5. Effect of restraint stress on heart rate in the presence of saline or DAMPGO in rats pretreated centrally 48 h earlier with either saline or β-FNA. Saline or DAMPGO was injected ICV 21 min before the onset of stress. Peak heart rate responses are shown at 1-2 min after the onset of restraint compared to the level immediately before restraint.

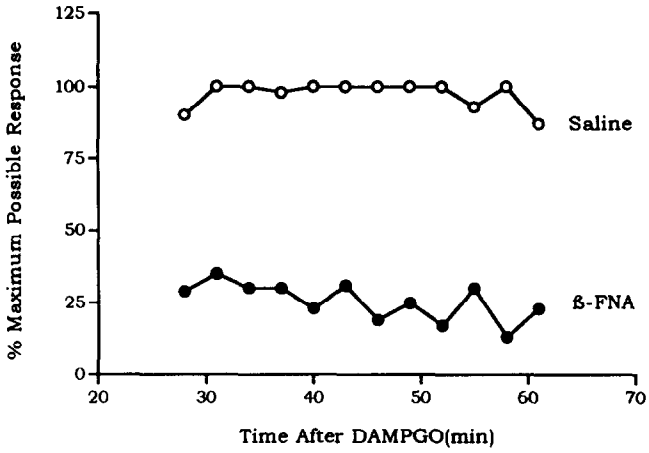

FIG. 6. Blockade of DAMPGO induced-antinociception by pretreatment with β-FNA. Tail-flick testing started 5 min after the onset of restraint stress, i.e., 26 min after ICV injection of DAMPGO. Rats were pretreated centrally 48 h earlier with either saline or β-FNA. Antinociception is shown as the percent of maximum possible response in a tail-flick test. Predrug response latencies (mean ± SE) for the saline and β-FNA pretreated group were 2.16 ± 0.1 and 2.44 ± 0.13 s, respectively. Analysis of variance revealed highly significant effect of β-FNA in inhibiting DAMPGO antinociception.

the heart is mediated by mu opioid receptors. The DAMPGO-induced bradycardia is due to mu opioid receptor activation of cardiac vagal outflow because pretreatment with atropine methyl nitrate converts the DAMPGO-induced bradycardia to a tachycardia (16). Pretreatment with β-FNA also inhibited the DAMPGO-induced increase in sympathoadrenal outflow as assessed by plasma catecholamine responses. A small increase in heart rate was observed in response to DAMPGO in the present study in rats pretreated with β-FNA. This suggests that mu opioid receptors can also produce an increase in sympathetic outflow to the heart. However, mu opioid receptor stimulation of the parasympathetic control of heart rate appears to predominate. The effect on sympathoadrenal function was not as profound as that on heart rate. Previous studies demonstrated that brain opioid effects on sympathoadrenal function are mediated predominantly by mu receptors with a smaller contribution by delta receptors (12).

Concurrent with the bradycardia and plasma catecholamine release, DAMPGO produces an increase in arterial blood pressure. β-FNA only slightly attenuated the blood pressure response to DAMPGO. Other studies have also demonstrated that DAMPGO and other mu-opioid agonists stimulate sympathetic outflow and produce hypertension (12,16,20,25). It is possible in our study that β-FNA may have failed to inactivate all the receptors accessible to DAMPGO that control blood pressure. Martin et al. (17) reported that only 40-50% of brain mu opiate receptors are alkylated following ICV administration of β-FNA, 40 nmol. Alternatively, β-FNA may selectively inactivate a subtype of mu opiate receptors which does not mediate BP responses.

Restraint stress in saline-treated rats evoked a significant increase in HR and plasma catecholamines with a mild increase in BP. In the presence of DAMPGO, stress produced a further fall in heart rate. This stress-induced bradycardia was also blocked by β-FNA. It has been shown that stress induces the release of endogenous opioid peptides (32). Thus, stress in

the presence of an opioid peptide may increase the concentration of mu opioids at mu opioid receptors, thus shifting the dose-response curve to the left and producing enhanced parasympathetic activation for any given dose of the peptide. The present findings suggest that endogenously released opioids act on mu opiate receptors to produce stress-induced bradycardia. Stress also produced a further increase in sympathoadrenal catecholamine release and a mild increase in blood pressure in rats treated with DAMPGO. Pretreatment with β-FNA, in the presence of DAMPGO, did not significantly alter these stress-induced increases in sympathetic outflow. This suggests that mu opioids are not solely responsible for changes in cardiovascular function during stress. Our data suggest that central mu opioid receptors are involved in parasympathetic outflow to the heart under basal and stressful conditions and in sympathoadrenal release of catecholamines. Reversal by restraint stress of mu-opioid-induced effects has also been shown in the regulation of body temperature (28). ICV administration of DAMPGO produced an increase in body temperature, whereas restraint stress imposed on DAMPGO-pretreated rats resulted in a fall in body temperature. These data suggest that in the presence of mu-opioid agonist, restraint stress may alter autonomic function, modulating not only the cardiovascular system but also temperature regulation.

Although the present study and other studies suggest that DAMPGO-induced antinociception and changes in HR, BP, and plasma catecholamine are mediated by pharmacologically similar populations of receptors, it is important to note that these effects are probably not mediated by the same population of receptors. In addition to anatomical distinctions, there are also pharmacological distinctions to be made between analgesia, cardiovascular responses, and catecholamine secretion induced by mu opioids. For example morphine-induced respiratory depression is antagonized by β-FNA (33,36) but not by naloxonazine (14), which has relatively high affinity for the mu-1 opiate receptor subtype. Findings such as these support the notion that subtypes of the mu receptors exist, and that these subtypes mediate distinct effects.

ACKNOWLEDGEMENTS

The authors would like to thank Frances Bobbitt for excellent technical assistance. These studies were supported in part by Tobacco and Health Research Institute, University of Kentucky and by the Veterans Administration.

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