Pharmacology Biochemistry & Behavior, Vol. 21, pp. 441–448, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

[D-Ala^2]-Methionine Enkephalinamide Reflexively Induces Antinociception by Activating Vagal Afferents[1]

ALAN RANDICH AND WILLIAM MAIXNER

Departments of Psychology and Pharmacology, The University of Iowa, Iowa City, IA 52242

Received 8 June 1984

RANDICH, A. AND W. MAIXNER. *[D-Ala^2]-methionine enkephalinamide reflexively induces antinociception by activating vagal afferents.* PHARMACOL BIOCHEM BEHAV 21(3) 441–448, 1984.—Experiment 1 showed that intravenous administration of [D-Ala^2]-methionine enkephalinamide resulted in dose-dependent inhibition of the tail-flick reflex, mild hypotension, and bradycardia. The enkephalinamide-induced inhibition of the tail-flick reflex and cardiovascular effects were eliminated in the bilateral cervical vagotomized anesthetized rat preparation, but were unaffected by either a unilateral right vagotomy or bilateral sinoaortic deafferentation in the conscious rat preparation. Experiment 2 demonstrated that the antinociceptive and cardiovascular actions of enkephalinamide were eliminated by pretreatment with intravenous administration of the opioid-receptor antagonist naloxone. These experiments strongly suggest that peripherally circulating enkephalins could reflexively induce analgesia by activating cardiopulmonary receptors whose afferents travel in the vagi.

Antinociception Vagus Enkephalinamide

ENKEPHALINS and enkephalin-like substances exist in peripheral tissues. Leucine- and methionine-enkephalin are stored in cardiac tissue [6] and both of these substances are contained within vesicles of the chromaffin cells of the adrenal medulla [18]. It has also been established that chromaffin cells synthesize leucine- and methionine-enkephalin, and that the enkephalins are co-stored and co-secreted with the catecholamines in the adrenal medulla (for review see [20]). Finally, enkephalin-like substances are released into the venous circulation following either physiological or pharmacological activation of renal sympathetic nerves [3,4].

Circulating enkephalins have been demonstrated to play a physiological role in cardiovascular shock (for review see [5]), stress-induced analgesia [7], and cardiorespiratory function [10, 21, 22]. However, it is generally held that circulating enkephalins would be unlikely to produce analgesia, because these substances do not readily cross the blood brain barrier and are rapidly degraded [11,17]. For example, Pert and her colleagues [12] failed to observe any analgesia following intravenous administration of 50 mg/kg of [D-Ala^2]-methionine-enkephalinamide (DALA), although only two subjects were used and no specific nociceptive time sampling data were provided. In contrast, Maixner and Randich [8] proposed that enkephalins released from peripheral sources may stimulate peripheral rather than central opioid receptors to induce analgesia by reflexively engaging vagal afferents linked to endogenous pain inhibition systems of the CNS. In this view, the failure of Pert *et al.* [12] to observe analgesia following administration of DALA may simply reflect an inadequate sampling procedure for detection of nociceptive changes which would be indicative of a vagal reflex mechanism. Several lines of evidence are consistent with this proposal and are considered in the following sections.

First, the activity of vagal afferents arising from receptors located in the cardiopulmonary region has been established to modify nociceptive responses to a variety of painful stimuli. Indirect evidence supporting this view derives from studies showing that electrical stimulation of vagal afferents attenuates the firing of low threshold, wide dynamic range and high threshold spinothalamic neurons projecting from laminae I, II, V, and VII of the spinal dorsal horn [1, 2, 19]. Conversely, resection of the right cervical vagal nerve trunk both reverses the opioid-mediated analgesia manifested by the Spontaneously Hypertensive Rat (SHR) in the hot-plate assay of pain sensitivity [9], and attenuates stress-induced analgesia (SIA) resulting from exposure to 30 min of intermittent foot-shock [8]. Direct evidence supporting a vagal influence on pain perception derives from recent work showing that activation of vagal afferents by (1) volume expansion results in long-lasting inhibition of the tail-flick reflex to painful radiant heat, and this effect is attenuated by right cervical vagotomy [8], and (2) administration of veratrine induces antinociception, and this effect is eliminated by bilateral cervical vagotomy but not by either right or left vagotomy alone [16]. The outcomes of the vagal resection studies also strongly suggest that volume expansion and veratrine admin-

[1]This research was supported by a grant from N.I.H. (NS 18341) to A. Randich.

istration activate different cardiopulmonary receptors, but that both operate through vagal afferents to inhibit responses to painful stimuli.

Second, peripherally administered enkephalins induce cardiovascular and respiratory changes by activating peripheral opioid receptors that are synthesized by and localized on vagal afferent fibers [10,23]. For example, administration of [D-Ala^2, Met^5]-enkephalinamide or [D-Ala^2, Leu^5]-enkephalinamide into the right atrium of decerebrate rats elicits bradycardia, a transient biphasic blood pressure response, and apnea within 1–2 sec of administration. These effects were abolished in animals with bilateral vagotomies [21]. Similarly, administration of morphine into the right atrium of decerebrate rats results in a similar triad of cardiorespiratory changes and is a consequence of stimulation of opiate receptors associated with pulmonary C-fibers, e.g., J-receptors [22].

In summary, activation of vagal afferents by either volume expansion or veratrine administration has been shown to inhibit responses to painful stimuli, while enkephalins clearly activate peripheral opioid receptors whose afferents travel in the vagi to induce cardiorespiratory changes. On the basis of this information, it need only be hypothesized that enkephalins might also activate peripheral opioid receptors with vagal afferents to reflexively induce antinociceptive responses. As noted previously, Maixner and Randich [8] proposed this view based upon differences obtained with both opioid receptor antagonist and vagal resection manipulations in volume expansion and SIA paradigms. Specifically, their data suggested that changes in blood volume and peripherally released enkephalins are capable of independently activating peripheral receptors (for example, opioid and stretch receptors), but that the afferents from both receptor types travel in the vagi and their activation induces antinociception.

The purpose of the present experiments was to test the view that peripherally administered [D-Ala^2]-methionine-enkephalinamide (DALA) is capable of inducing antinociception by activating cardiopulmonary receptors with vagal afferents. The term antinociception will be used in deference to the term analgesia when discussing the data of the present experiments, since many factors other than analgesia can produce changes in responding to nociceptive stimuli, e.g., inhibition of motor activity.

EXPERIMENT 1

In Experiment 1, the nociceptive (tail-flick reflex) and cardiovascular (arterial blood pressure and heart rate) effects of intravenously administered DALA were assessed in rats with sham-operations, unilateral right cervical vagotomies, bilateral sinoaortic deafferentations (SADs), or bilateral cervical vagotomies. The sham-operation group was used to establish the control dose-response functions. The unilateral cervical vagotomy, bilateral SAD, and bilateral cervical vagotomy groups were used to determine the potential role of vagal and sinoaortic afferents in mediating the physiological actions of DALA. DALA was used since it is a relatively long-lasting enkephalin analogue that is reasonably well characterized with respect to permeability of the blood-brain barrier. For example, the estimated first pass extraction fraction ((permeability constant × capillary surface area)/(mean regional blood flow)) is 2.6% which indicates a low and nonspecific permeability of the blood-brain barrier and one similar to that observed to other neurotransmitters [11].

METHOD

Subjects

Twenty-five male Sprague-Dawley rats obtained from Hormone Assay Laboratories in Chicago served as subjects. The rats were individually housed in wire-mesh cages under a 12:12 hr light-dark cycle. Food and water were available on an ad lib basis.

Apparatus

Nociceptive responses were measured with a tail-flick apparatus. The radiant heat stimulus was provided by a 500 W projector bulb housed in a metal casing and focused on the rats' tail through a small opening in the metal housing. Onset and termination of each trial were controlled automatically by a digital timer. The intensity of the radiant heat stimulus, which was constant for all subjects, was intended to produce a tail-flick response of approximately 3–4 sec in a normal rat.

Arterial blood pressure and heart rate were recorded on a Beckman R11A rectilinear dynagraph from the signal provided by a Century pressure transducer.

Surgical Techniques

Since the rat cannot withstand bilateral vagotomy for any substantial period of time, the unilateral vagotomies and bilateral SADs were carried out at a different time than bilateral vagotomies and under different experimental protocols with their own controls. Rats in the right vagotomy (N=5) and sham vagotomy (N=5) groups were anesthetized with ether, whereas rats in the bilateral SAD group (N=5) were anesthetized with pentobarbital sodium (50 mg/kg) and underwent the appropriate surgical treatment four weeks prior to testing. Rats in the right vagotomy condition received a 3-cm midventral incision in the cervical region. Dissection continued until the underlying sternocleidomastoid group was exposed. The right sternomastoid was displaced laterally and the deeper omohyoid was cut at right angles to its fiber direction. The right vagus was located and separated from the right common carotid artery and other surrounding tissues and nerves. The vagus was then cut and a 1 cm segment removed inferior to the superior laryngeal nerve. Rats in the sham vagotomy condition received the procedure described above for right vagotomy, but the vagus was not cut. Rats in the bilateral SAD condition received the same basic surgical procedure as rats in the right vagotomy group, except that the superior laryngeal nerves were resected and the sympathetic trunks were resected caudal to the superior cervical ganglion. Denervation of the carotid sinus baroreceptors was accomplished by stripping the bifurcation of all fibers and then painting the region with a solution of 10% phenol in ethanol. The integrity of the SAD was confirmed following termination of the experiment. This was accomplished by administering a bolus injection of phenylephrine sufficient to produce an increase in arterial blood pressure of approximately 40 mmHg. The failure to observe reflex bradycardia of greater than 12 beats per min indicated a successful SAD.

Four weeks later, these rats were instrumented with arterial and venous cannulae. Each rat was anesthetized with pentobarbital sodium (50 mg/kg). An incision was made in the pectoral region 1 cm to the right of the midventral line and extending 2 cm craniad from the pectoralis. The external jugular vein was exposed at its junction with the right subclavian vein and the connective tissue surrounding the area was cleared. A cannula (Silastic) was inserted caudad

through the brachiocephalic vein approximately 4 cm. The cannula was anchored to adjacent tissue.

A 3-cm midventral incision was then made in the cervical region. Dissection continued until the underlying sternocleidomastoid group was exposed. The left sternomastoid was reflected laterally and the deeper omohyoid was cut at right angles to its fiber direction. The left common carotid artery was then separated from the surrounding tissues and nerves. A cannula (Microline) was advanced caudad 3–4 cm and anchored to adjacent muscles. The arterial and venous cannulae were then drawn subcutaneously around the neck and exited through a dorsal incision. The cannulae were anchored to the neck and flushed with a saline-heparin solution. Twenty-four hr later they were tested in the manner described in the testing section to follow.

Rats in the bilateral vagotomy (N=5) and sham bilateral vagotomy (N=5) groups were anesthetized with pentobarbital sodium (50 mg/kg) and received implantation of the arterial and venous cannulae as described previously. Approximately 40–45 min following the administration of the anesthetic, the vagi were resected in the bilateral vagotomy group, whereas the nerves were merely exposed in the sham bilateral vagotomy group. The rats were then immediately tested in the lightly anesthetized state using the procedure described in the testing section to follow.

Testing

Each rat was placed in a Plexiglas restraining tube and the cannulae were connected. Tail-flick trials were then administered until baseline latencies were between 3–4 sec and remained stable from trial to trial. Following stabilization of the tail-flick response, each rat received successive bolus I.V. infusions of isotonic saline, 5 μg/kg, 50 μg/kg, and 500 μg/kg of [D-Ala²]-methionine enkephalinamide (DALA—Sigma Co.). DALA was dissolved in sterile isotonic saline. Drug solutions and saline vehicle were infused in a volume 1 ml/kg at a rate of approximately 200 μl/sec. Each saline or drug administration was followed by a 400 μl saline flush. Tail-flick trials were administered 0.25-, 1-, 2-, and 3-min after each drug dose. Arterial blood pressure and heart rate were continuously recorded. Approximately 10 min intervened between administration of each successive drug dose, but trials were never initiated unless baseline tail-flick latencies had returned to normal. A maximum latency of 10 sec was arbitrarily used to prevent tissue damage to the tail. Rats tested in the anesthetized preparation (bilateral and sham bilateral vagotomies) received essentially the same procedure with the following exceptions. First, these rats were tested within 3 min of resection of the vagi since this operation results in rapid death in the rat. Second, only the largest dose of DALA was administered (500 μg/kg). This was done to achieve the maximal antinociceptive effect within the limited time-frame available for testing these animals.

Data Analysis

Each tail-flick latency was converted to a tail-flick index by the equation ((test trial latency − baseline latency)/(10 sec − baseline latency) × 100)). The baseline latency was obtained by using the trial immediately preceding drug administration. A new baseline value was established following each drug administration. In addition to the cardiovascular measures obtained at the 0.25-, 1-, 2-, and 3-min trial points, the largest change in cardiovascular function manifested prior to the 0.25-min trial point was recorded and defined as the "Peak" response. Baseline tail-flick latencies, arterial pressures, and heart rates are presented for comparison purposes. Tail-flick indices, percentage change from baseline blood pressure, and percentage change from baseline heart rate (where peak responses are also included for cardiovascular measures) were subjected to analysis of variance. In each analysis in which the overall null hypothesis was rejected, Scheffe post-hoc comparisons were performed on the means. Alpha was set at 0.05.

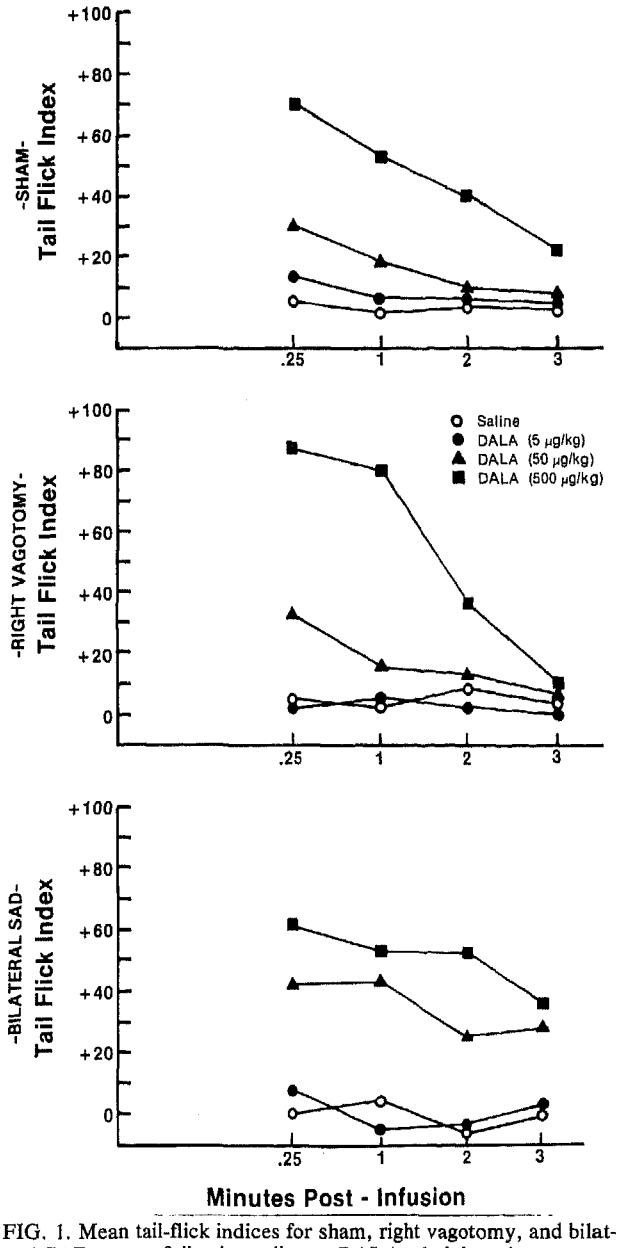

FIG. 1. Mean tail-flick indices for sham, right vagotomy, and bilateral SAD groups following saline or DALA administration.

RESULTS

Mean tail-flick indices are presented in Fig. 1 as a function of sham operation, right vagotomy, and bilateral SAD in

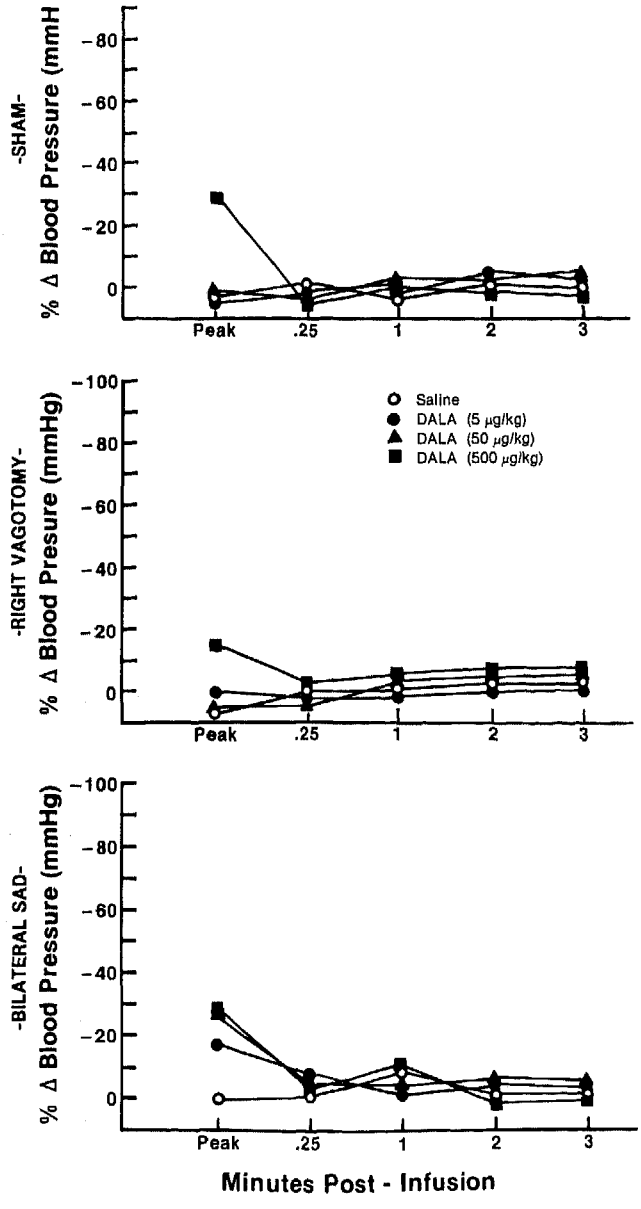

FIG. 2. Mean arterial blood pressures expressed as percent change from baseline for sham, right vagotomy, and bilateral SAD groups following saline or DALA administration.

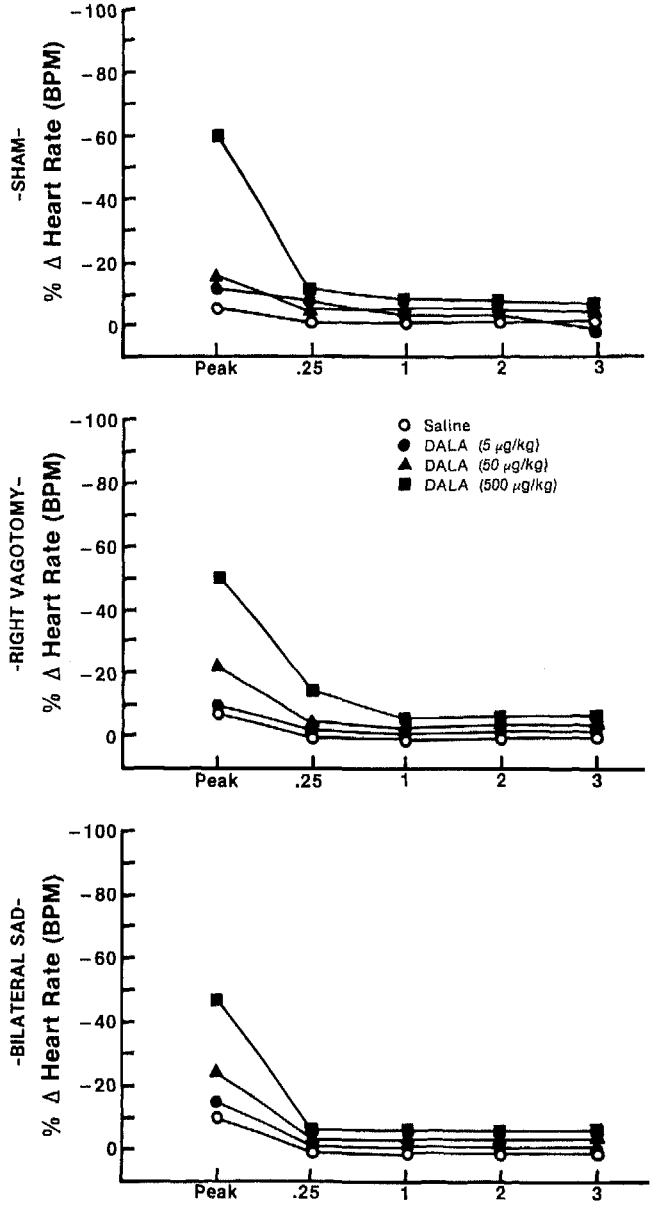

FIG. 3. Mean heart rates expressed as percent change from baseline for sham, right vagotomy, and bilateral SAD groups following saline or DALA administration.

the conscious rat preparation. Mean baseline tail-flick latencies obtained by collapsing across all baseline values during a test session were 3.72, 3.43, and 3.88 sec for groups sham operation, right vagotomy, and bilateral SAD, respectively. In general, Fig. 1 reveals that administration of DALA inhibits the tail-flick reflex at the 50 and 500 μg/kg doses, and that there was no major effect of either right vagotomy or bilateral SAD on tail-flick indices compared to sham controls. This was confirmed by an ANOVA of tail-flick indices which indicated no significant effect of the operation, $F(2,16)=0.05$; a significant effect of drug dose, $F(3,48)=58.29$; a significant effect of time, $F(3,48)=13.33$; and a significant dose × time interaction, $F(9,144)=6.42$. All other effects were not significant. Since the dose × time interaction was significant, the dose effect was analyzed by performing individual ANOVAs at each of the test trial time points with appropriate post-hoc analyses of dose means. The post-hoc analyses indicated that the mean tail-flick index for the 500 μg/kg dose differed significantly from saline at the 0.25-, 1-, 2-, and 3-min points, F's of 35.17, 22.19, 9.89, and 2.87, respectively, while the mean tail-flick indices for the 50 μg/kg dose differed significantly from saline at only the 0.25-min point, $F=3.82$. All other comparisons were not significant.

Figure 2 presents mean arterial blood pressures as a function of the percentage change from baseline values for the various operations. Mean baseline arterial pressures obtained by collapsing across all baseline values during a test

session were 111, 116, and 132 mmHg for groups sham operation, right vagotomy, and bilateral SAD, respectively. In general, administration of DALA results in little if any hypotension and analogous to the tail-flick data there was little effect of either right vagotomy or bilateral SAD on arterial blood pressure. This was confirmed by an ANOVA of arterial blood pressures indicating that only the time effect was significant, $F(4,64)=3.66$.

Figure 3 presents the mean heart rates as a function of percentage change from baseline values for the operations. Mean baseline heart rates obtained by collapsing across all baseline values during a test session were 489, 512, and 535 BPM for groups sham operation, right vagotomy, and bilateral SAD, respectively. In general, this figure indicates that DALA induces substantial bradycardia, and this response was observed within 1–2 sec of drug administration. However, the magnitude of this effect was not markedly altered by either right vagotomy or bilateral SAD. This view was supported by an ANOVA of heart rates which indicated no significant effect of operation, $F(2,16)=0.04$; a significant effect of drug dose, $F(3,48)=26.83$; a significant effect of time, $F(4,64)=80.40$; and a significant interaction of drug dose × time, $F(12,192)=29.52$. Since the dose × time interaction was significant, the dose effect was analyzed by performing individual ANOVAs at each of the test trial time points with appropriate post-hoc analyses of dose means. The post-hoc analyses indicated that the mean heart rates for the 500 μg/kg dose differed significantly from saline at the peak and 0.25-min points, F's of 34.07 and 5.08. All other comparisons were not significant.

In contrast to the failure of either right vagotomy or bilateral SAD to affect the antinociception produced by administration of DALA, the top panel of Fig. 4 reveals that bilateral vagotomy markedly attenuates DALA-induced inhibition of the tail-flick reflex in the anesthetized rat preparation. This was confirmed by an ANOVA of tail-flick indices indicating significantly greater tail-flick indices in sham-operated compared to bilateral vagotomized rats, $F(1,8)=8.23$; and a significant effect of time, $F(3,24)=3.71$. Moreover, a follow-up randomized block ANOVA on tail-flick latencies obtained both prior to (baseline latency) and during the test trial of bilateral vagotomized rats was not significant, $F(4,16)=2.40$, suggesting that no antinociceptive response occurred in these rats following DALA administration. The mean baseline tail-flick latencies were 4.28 and 4.05 sec for groups bilateral vagotomy and sham vagotomy, respectively.

The middle panel of Fig. 4 presents mean arterial blood pressures evoked by administration of DALA expressed as percentage change from baseline values. Clearly, bilateral vagotomy also abolished the hypotension evoked by DALA. An ANOVA of changes in arterial blood pressures indicated significantly greater hypotension in the sham-operated rats, $F(1,8)=8.85$; a significant effect of time, $F(4,32)=8.56$; and a significant interaction of operation × time, $F(4,32)=8.41$. The operation × time interaction suggests that in the absence of vagal control, DALA administration produces a modest pressor response. The mean baseline arterial pressures were 132 and 122 mmHg for groups bilateral vagotomy and sham vagotomy, respectively.

Finally, mean heart rates are presented in the bottom panel of Fig. 4 expressed as a function of percentage change from baseline. Again, bilateral vagotomy completely abolished the bradycardic action of the drug. This was confirmed by an ANOVA of heart rates indicating significantly lower heart rates in the sham-operated rats, $F(1,8)=69.53$; a

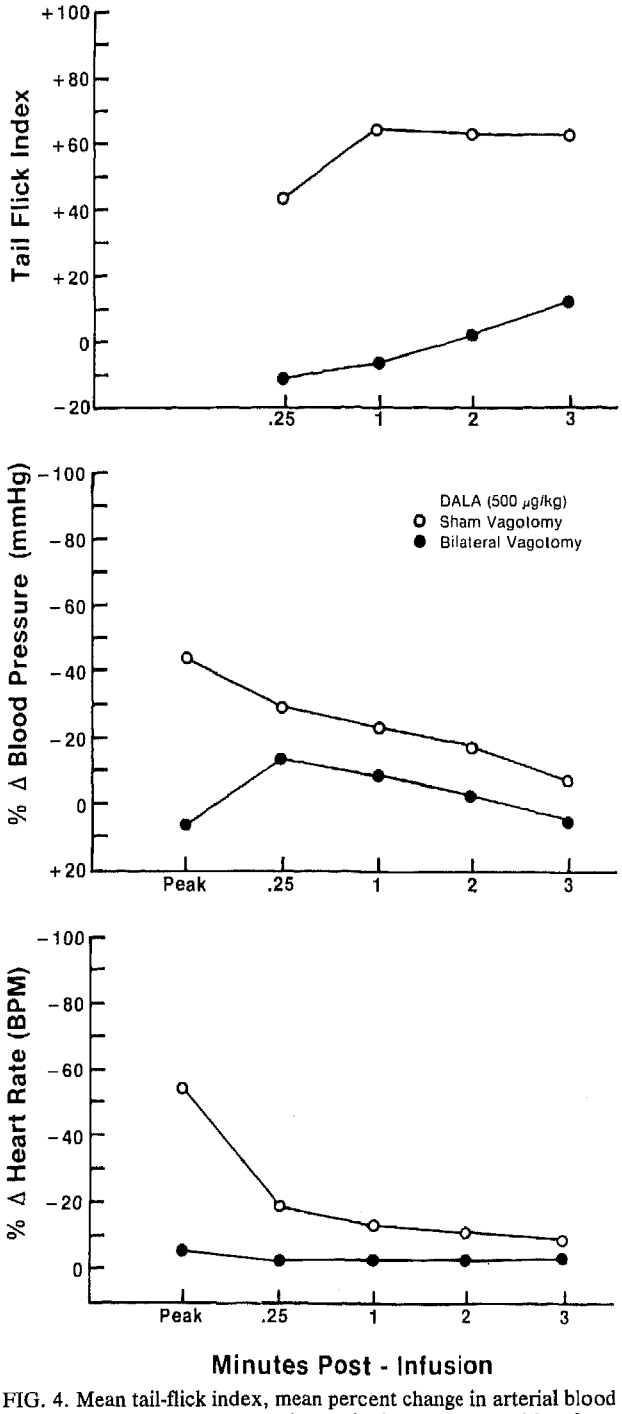

FIG. 4. Mean tail-flick index, mean percent change in arterial blood pressure, and mean percent change in heart rate resulting from DALA administration (500 μg/kg) in bilateral vagotomized rats and sham controls.

significant effect of time, $F(4,32)=22.25$; and a significant interaction of operation × time, $F(4,32)=17.35$. Mean baseline heart rates were 444 and 474 BPM for groups bilateral vagotomy and sham vagotomy, respectively.

In summary, Experiment 1 demonstrates that the inhibition of the tail-flick reflex, bradycardia, and hypotension in-

duced by intravenous administration of DALA is a consequence of activation of peripheral receptors whose afferents travel in the vagi. Unilateral resection of the right vagal nerve trunk is not sufficient to alter these effects nor do high pressure baroreceptors appear to mediate these effects since bilateral SAD was generally without influence.

EXPERIMENT 2

The purpose of Experiment 2 was to determine whether administration of the opioid receptor antagonist naloxone would abolish the antinociception induced by intravenous administration of DALA. This outcome would indicate that DALA was producing a relatively specific effect on an opioid receptor, rather than a non-specific drug action.

METHOD

Subjects

Ten male Sprague-Dawley rats served as subjects. All other conditions were as described in Experiment 1.

Apparatus

The apparatus was as described in Experiment 1.

Surgical Techniques

All rats received implantation of arterial and venous cannulae as described in Experiment 1.

Testing

Twenty-four hr later, all rats were tested in the tail-flick assay in the conscious state. Five control rats were tested 0.25-, 1-, 2-, and 3-min following successive administration of isotonic saline and 500 μg/kg of DALA. These data served as control values. Five experimental rats were tested in a similar manner following successive administration of saline, 1 mg/kg of naloxone HCl (NIDA), and 500 μg/kg of DALA. It should be noted that following the naloxone test trial a supplemental 1 mg/kg dose of naloxone was administered 5 min prior to the DALA trial. These data allowed determination of the effect of naloxone alone and its action on nociceptive and cardiovascular effects of DALA. As in Experiment 1, all drugs were dissolved in sterile isotonic saline. Drug solutions and saline vehicle were infused in a volume of 1 ml/kg and at a rate of approximately 200 μl/sec. The arterial pressure and heart rate data for one animal in the control condition were not scorable, and the arterial pressure data for one animal in the naloxone condition was not scorable. These data were not analyzed.

RESULTS

Mean tail-flick indices obtained for Experiment 2 are presented in Fig. 5. The top panel of Fig. 5 shows the typical antinociceptive action of 500 μg/kg of DALA on the tail-flick reflex. A separate ANOVA on these data indicated a significant antinociceptive action of DALA compared to saline, $F(1,4)=7.65$; a significant effect of time, $F(3,12)=5.66$; but no significant interaction of drug × time. Mean baseline tail-flick latencies were 2.89 and 3.28 sec prior to the saline and DALA trials, respectively. The bottom panel of Fig. 5 shows that pretreatment with naloxone completely eliminates the normal antinociceptive action of DALA. This was confirmed by an ANOVA of tail-flick indices which indicated only a significant effect of the drug, $F(2,8)=5.37$. Post-hoc comparisons of mean tail-flick indices obtained by collapsing across time indicated that the means of saline and 500 μg/kg of DALA did not differ, $F=0.05$, but that the means of saline and 500 μg/kg of DALA combined differed significantly from 1 mg/kg of naloxone, $F=5.31$. Thus, tail-flick indices were smaller following administration of naloxone compared to either saline or DALA. However, a follow-up randomized block ANOVA of tail-flick latencies obtained prior to (baseline latency) and during the naloxone test trial indicated no significant effect, $F(4,16)=0.89$, suggesting that no hyperalgesia resulted from administration of naloxone alone. Mean baseline tail-flick latencies were 3.06, 3.67, and 2.94 sec prior to the saline, naloxone, and DALA trials, respectively.

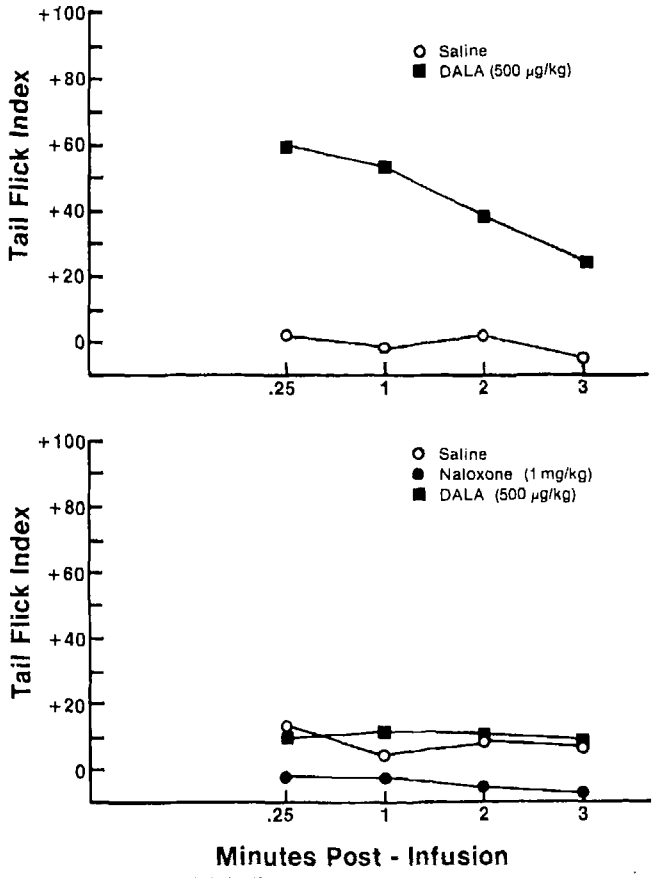

FIG. 5. Mean tail-flick indices following DALA administration (500 μg/kg) in either the absence (top panel) or the presence (bottom panel) of naloxone.

Similar presentations of the arterial blood pressure and heart rate data are shown in Figs. 6 and 7. In general, DALA evokes a hypotensive and bradycardic response in the absence of naloxone, but does not evoke either response in the presence of naloxone. An ANOVA on arterial blood pressures in the absence of naloxone indicated only a significant effect of time, $F(4,12)=6.72$; and a significant interaction of drug × time, $F(4,12)=18.38$. Mean baseline arterial pressures were 129 and 127 mmHg prior to the saline and DALA trials, respectively. An ANOVA on arterial blood pressures in the presence of naloxone indicated no significant effects. Mean baseline arterial pressures were 118, 118, and 118

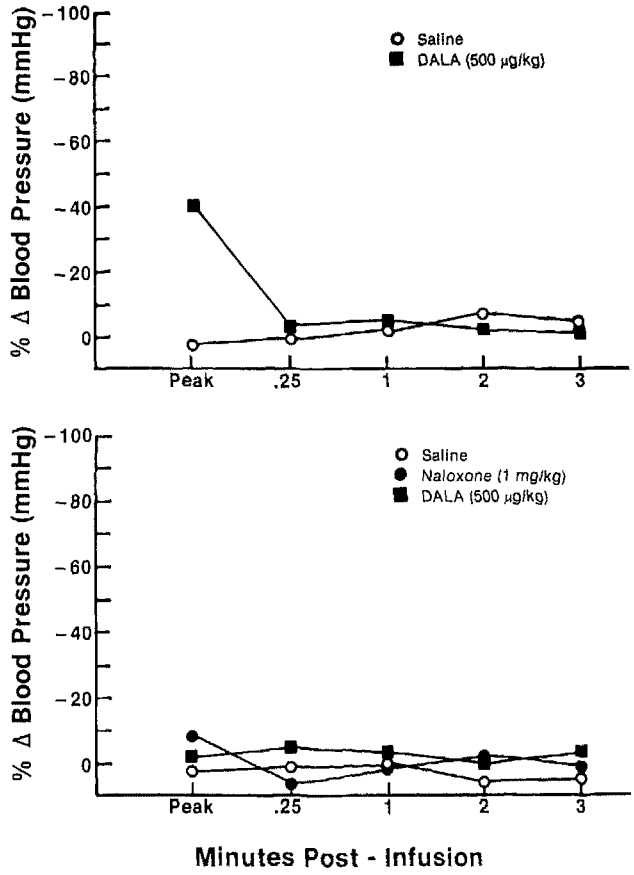

FIG. 6. Mean arterial blood pressures expressed as percent change from baseline following DALA administration (500 μg/kg) in either the absence (top panel) or the presence (bottom panel) of naloxone.

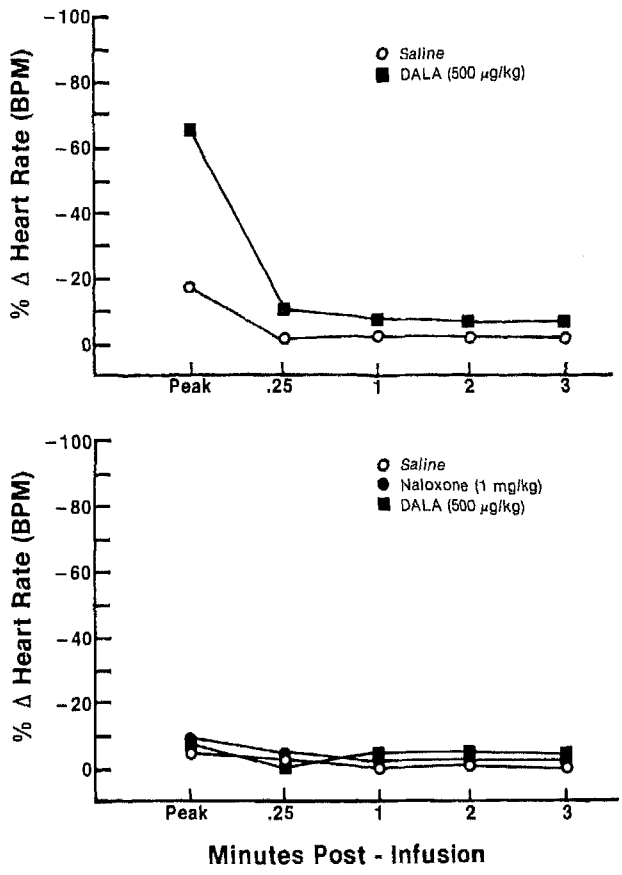

FIG. 7. Mean heart rates expressed as percent change from baseline following DALA administration (500 μg/kg) in either the absence (top panel) or the presence (bottom panel) of naloxone.

mmHg prior to the saline, naloxone, and DALA trials, respectively. Similarly, an ANOVA of heart rates in the absence of naloxone indicated a significant effect of drug, $F(1,3)=12.57$; a significant effect of time, $F(4,12)=65.61$; and a significant interaction of drug × time, $F(4,12)=6.18$. Mean baseline heart rates were 537 and 520 BPM prior to saline and DALA trials, respectively. However, an ANOVA of heart rates in the presence of naloxone indicated only a significant effect of time, $F(4,16)=3.18$. Mean baseline heart rates were 528, 528, and 514 BPM prior to saline, naloxone, and DALA trials, respectively. In summary, naloxone completely eliminates the nociceptive and cardiovascular changes induced by administration of 500 μg/kg of DALA.

GENERAL DISCUSSION

The present findings are consistent with the general view that cardiopulmonary visceral afferents, which historically have only been associated with cardiovascular regulation, are also important regulators of somatosensory function [8, 9, 13, 14, 15]. Specifically, intravenous administration of DALA induces inhibition of the tail-flick reflex, bradycardia, and mild hypotension. These nociceptive and cardiovascular changes are most likely a consequence of DALA stimulation of cardiopulmonary opioid receptors with associated vagal afferents because they are abolished by either pretreatment with naloxone or bilateral cervical vagotomy. The observation that unilateral cervical vagotomy fails to affect nociceptive and cardiovascular actions of DALA suggests that the activation of a single vagal nerve trunk is sufficient to sustain these reflex responses. Moreover, the failure of bilateral SAD to alter these effects of DALA indicates that sinoaortic baroreceptors play no significant mediating role in these phenomena.

All of the cardiovascular findings are comparable to those reported previously by Willette and Sapru [21,22] who used different met- and leu-enkephalinamide analogues in the decerebrate rat preparation. That is, the bardycardia and hypotension appear to be mediated by activation of opioid receptors in the cardiopulmonary region because these responses occurred within 1–2 sec of drug infusion and were abolished by bilateral cervical vagotomy. The implications of such cardiovascular responses in cardiovascular shock, asthmatic attacks, and sudden death associated with opioid use have been considered elsewhere [5, 10, 21, 22].

The novel finding that DALA induces a reflex antinociceptive effect, which depends upon the integrity of at least one vagal nerve trunk, suggests a potential mechanism by which circulating enkephalins could induce analgesia when released from various peripheral sources. As noted previously, endogenous opioid-like substances are synthesized in, stored in, and released from peripheral sites. There is also good evidence that these substances serve many physiological roles (for review see [5]). The implica-

tion of the present data is that circulating opioid-like substances may induce analgesia by a peripheral vagal reflex mechanism and/or by an effect on the CNS, where the latter will depend upon their capacity to pass the blood-brain barrier or to operate at fenestrated areas. In the present experiments, DALA appears only to exert a vagal reflex effect with little or no direct effect on the CNS within the time parameters studied. In agreement with this contention, peripherally administered DALA shows little ability to cross the blood-brain barrier [11]. However, this would not preclude the capacity of another circulating endogenous opioid-like substance from producing analgesia either by a central effect only or by both peripheral and central actions. That is, all possible combinations of peripheral and central actions are now plausible, although dependent upon the physical-chemical properties of the opioid receptor agonist and its' specificity for the various opioid receptor complexes associated with analgesia. It should be emphasized, however, that the present data do not permit the conclusion that the observed nociceptive and cardiovascular phenomena necessarily represent endogenous physiological responses, since relatively large amounts (μg/kg) of DALA were required. On the other hand, the actual amount that circulates under physiological conditions is unknown because these opioid-like substances appear to be secreted in multiple and protected forms from both the adrenal medulla and cardiac tissues [3, 4, 5, 6, 20]. Thus, it is possible that the amount available to peripheral vagal opioid receptors after intra-atrial administration may be very comparable to that amount which is present under physiological conditions. It remains an open issue to what extent circulating β-endorphin and β-endorphin-like substances derived from pituitary sources might also interact with this vagal reflex mechanism.

Finally, Randich and Maixner [15] proposed that peripheral and central systems involved in cardiovascular regulation are physiologically linked to systems involved in pain perception to form a functional network governing the elaboration of adaptive responses to physical and psychological stressors. Although the present study did not demonstrate that the vagal afferents mediating the cardiopulmonary baroreceptor reflex are the same afferents as those mediating inhibition of the tail-flick reflex, we have never been able to dissociate these two reflexes in our previous work [8, 15, 16] which is at least indirectly supportive for a common afferent pathway. Further, this network may be critical for the full expression of SIA, stimulation-produced analgesia (SPA), and acupuncture analgesia. Specifically, these experimentally-induced analgesias may result either from the physiological activation of vagal afferents by increases in central venous pressure or from the resulting secretion of humoral substances into the circulation, which in turn stimulates vagal afferents. We feel the observations presented in this report support such a conceptualization.

ACKNOWLEDGEMENTS

We thank R. L. Fisher and L. Morton for their help in conducting this research, and Dr. R. T. Ross for helpful comments on an earlier version of this manuscript. We also thank NIDA for their generous contribution of naloxone hydrochloride.

REFERENCES

1. Ammons, W. S., R. W. Blair and R. D. Foreman. Vagal afferent inhibition of primate thoracic spinothalamic neurons. *J Neurophysiol* **50:** 926–939, 1983.
2. Ammons, W. S., R. W. Blair and R. D. Foreman. Vagal afferent inhibition of spinothalamic cell responses to sympathetic afferents and bradykinin in the monkey. *Cir Res,* in press, 1984.
3. Govoni, S., I. Hanbauer, T. D. Hexum, H.-T. Yang, G. D. Kelly and E. Costa. In vivo characterization of the mechanisms that secrete enkephalin-like peptides stored in dog adrenal medulla. *Neuropharmacology* **20:** 639–645, 1981.
4. Hanbauer, I., S. Govoni, E. A. Majane, H.-T. Yang and E. Costa. In vivo regulation of release of enkephalin-like peptides from dog adrenal medulla. In: *Regulatory Peptides: Functional and Pharmacological Aspects,* edited by M. Trabucchi and E. Costa. New York: Raven Press, 1982, pp. 209–215.
5. Holaday, J. W. Cardiovascular effects of endogenous opiate systems. *Annu Rev Pharmacol Toxicol* **23:** 541–594, 1983.
6. Lang, R. E., K. Hermann, R. Dietz, W. Gaida, D. Ganten, K. Kraft and Th. Unger. Evidence for the presence of enkephalins in the heart. *Life Sci* **32:** 399–406, 1983.
7. Lewis, J. W., M. G. Tordoff, J. E. Sherman and J. C. Liebeskind. Adrenal medullary enkephalin-like peptides may mediate opioid stress-induced analgesia. *Science* **217:** 557–559, 1982.
8. Maixner, W. and A. Randich. Role of the right vagal nerve trunk in antinociception. *Brain Res* **298:** 374–377, 1984.
9. Maixner, W., K. B. Touw, M. J. Brody, G. F. Gebhart and J. P. Long. Factors regulating the altered pain perception in the spontaneously hypertensive rat. *Brain Res* **237:** 137–145, 1982.
10. McQueen, D. S. Opioid peptide interactions with respiratory and circulatory systems. *Br Med Bull* **39:** 77–82, 1983.
11. Pardridge, W. M. and L. J. Mietus. Enkephalin and blood-brain barrier: Studies of binding and degradation in isolated brain microvessels. *Endocrinology* **109:** 1138–1143, 1981.
12. Pert, C. B., A. Pert, J.-K. Chang and B. T. W. Fong. [D-Ala^2]-met-enkephalinamide: A potent, long-lasting synthetic pentapeptide analgesic. *Science* **194:** 330–332, 1976.
13. Randich, A. Sinoaortic baroreceptor reflex arc modulation of nociception in spontaneously hypertensive and normotensive rats. *Physiol Psychol* **10:** 267–272, 1982.
14. Randich, A. and C. Hartunian. Activation of the sinoaortic baroreceptor reflex arc induces analgesia: Interactions between cardiovascular and endogenous pain inhibition systems. *Physiol Psychol* **11:** 214–220, 1983.
15. Randich, A. and W. Maixner. Interactions between cardiovascular and pain regulatory systems. *Neurosci Biobehav Rev* **8:** in press, 1984.
16. Randich, A., T. A. Simpson, P. A. Hanger and R. L. Fisher. Activation of vagal afferents by veratrine induces antinociception. *Physiol Psychol,* submitted, 1984.
17. Rapoport, S. I., W. A. Klee, K. D. Pettigrew and K. Ohno. Entry of opioid peptides into the central nervous system. *Science* **207:** 84–86, 1980.
18. Schultzberg, M., J. M. Lundberg, T. Hökfelt, L. Terenius, J. Brandt, R. P. Elde and M. Goldstein. Enkephalin-like immunoreactivity in gland cells and nerve terminals of the adrenal medulla. *Neuroscience* **3:** 1169–1186, 1978.
19. Theis, R. and R. D. Foreman. Descending inhibition of spinal neurons in the cardiopulmonary region by electrical stimulation of vagal afferent nerves. *Brain Res* **207:** 178–183, 1981.
20. Viveros, O. H. and S. P. Wilson. The adrenal chromaffin cell as a model to study the co-secretion of enkephalins and catecholamines. *J Autonom Nerv Syst* **7:** 41–58, 1983.
21. Willette, R. N. and H. N. Sapru. Pulmonary opiate receptor activation evokes a cardiorespiratory reflex. *Eur J Pharmacol* **78:** 61–70, 1982.
22. Willette, R. N. and H. N. Sapru. Peripheral versus central cardiorespiratory effects of morphine. *Neuropharmacology* **21:** 1019–1026, 1982.
23. Young, W. S., J. K. Wamsley, M. A. Zarbin and M. J. Kuhar. Opiate receptors undergo axonal flow. *Science* **210:** 76–77, 1980.