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Cardiovascular Effects of Dynorphin A (1-13) in Conscious Rats and its Modulation of Morphine Bradycardia Over Time

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GLATT, C. E., J. R. KENNER, J. B. LONG AND J. W. HOLADAY. *Cardiovascular effects of dynorphin A (1-13) and its modulation of morphine bradycardia over time*. PEPTIDES 8(6) 1089-1092, 1987.—The short-term cardiovascular effects of dynorphin A (1-13), as well as its effects upon morphine bradycardia were investigated. In unanesthetized, unrestrained rats, intracerebroventricular (ICV) dynorphin A (1-13) injections (10–20 μ g) produced a dose-related pressor effect, whereas intravenous (IV) dynorphin A (1-13) (1.0 mg/kg) produced a depressor effect; these responses persisted less than five min. Heart rate was not significantly altered by these doses or routes of administration. Dynorphin A (1-13) also produced behavioral effects in the unanesthetized animals, such as wet dog shakes in response to IV administration and wet dog shakes accompanied by barrel rolling in response to ICV administration. To evaluate the effects of dynorphin A (1-13) pretreatment on the bradycardic response to IV morphine, rats were pretreated with 10 μ g dynorphin A (1-13) ICV four, six or eight hours prior to challenge with morphine sulfate (0.1 mg/kg IV). Four hour pretreatment with dynorphin A (1-13) (tested at 14:00 hr) resulted in a potentiation of morphine bradycardia, with six hours pretreatment (tested at 16:00 hr) no effect was observed, and eight hours following dynorphin A (1-13) pretreatment (tested at 18:00 hr) morphine bradycardia was attenuated. Additionally, the bradycardic response to IV morphine alone became more exaggerated as rats approached their nocturnal activity cycle. These data further establish that dynorphin A (1-13) exerts a potent, long lasting modulatory effect on morphine bradycardia and emphasize the importance of circadian variables in altering the magnitude of cardiovascular responses to opioid agonists.

Dynorphin A (1-13) Morphine bradycardia Circadian rhythms Multiple opiate receptors
Opiate receptor interactions Cardiovascular function

PHARMACOLOGICAL studies with alkaloid and peptide opioid agonists and antagonists have revealed a confusing array of cardiovascular responses that differ according to the opioid ligand used, state of consciousness of experimental animals, site(s) or routes of injection, doses, species, experimental stressors and other variables [6, 7, 17]. Of the many endogenous opioid peptides described to date, the autonomic effects of dynorphin-related peptides are the least studied. Although the short-term cardiovascular effects of dynorphin have been investigated in anesthetized rats following microinjections into discrete brain nuclei [2], as of yet the cardiovascular responses evoked by dynorphin A (1-13) have not been systematically compared following different routes of injection in unanesthetized rats. Therefore, an initial purpose of the present study was to define the short-term cardiovascular actions of dynorphin A (1-13) following its intracerebroventricular (ICV), intravenous (IV) or sub-

cutaneous (SC) injection in unanesthetized rats.

Recent evidence has accumulated to indicate that pretreatment with opioid agonists can subsequently influence the responses to other opioid agonists or antagonists. For example, data obtained from analgesic and binding assays has demonstrated that dynorphin A (1-13) is a kappa agonist with long-lasting μ antagonist properties [4, 14, 19]. Using anesthetized rats subjected to concomitant surgical stress, Kiang and Wei [12,13] have conducted a series of studies demonstrating that, depending upon the time of pretreatment, the SC injection of dynorphin A (1-13) can either potentiate or antagonize the short-term bradycardic effects of the μ agonist morphine. However, the confounding effects of anesthetics and routes of injection were not addressed in those studies.

Since the short-term bradycardic response to IV morphine is peripherally mediated [12,13], SC dynorphin A

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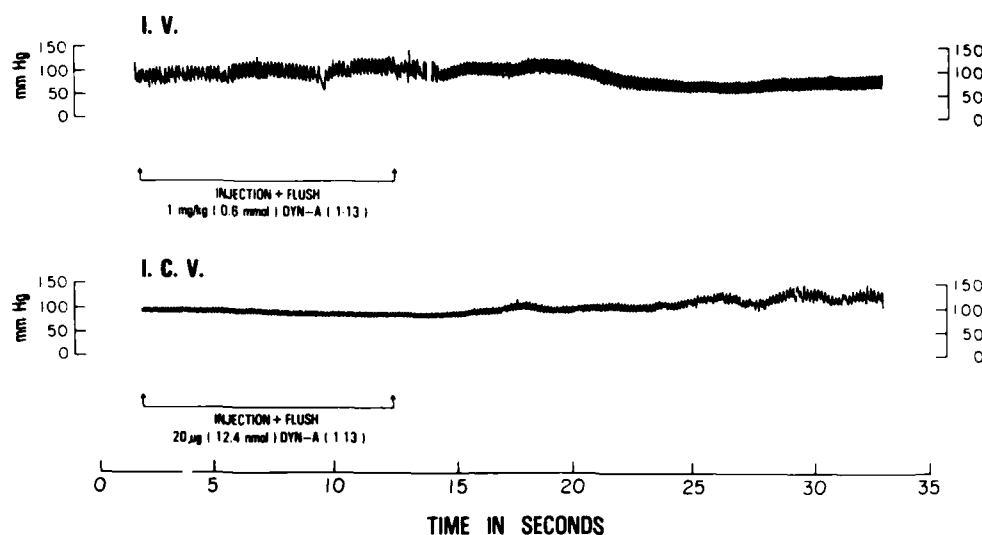


FIG. 1. (A) Physiograph trace of unanesthetized rat during IV administration of dynorphin A (1-13) 1 mg/kg (0.6 μ mol). Treatment resulted in tachycardia as well as hypotension. IV dynorphin also induced the behavioral effect of wet dog shakes (middle of trace). (B) Physiograph trace of unanesthetized rat during ICV administration of dynorphin A (1-13) 20 μ g (12.4 nmol). Treatment resulted in tachycardia as well as hypertension. ICV dynorphin also induced the behavioral effects of wet dog shakes and barrel rolling (right hand side of trace).

(1-13) pretreatment may modify morphine's bradycardic effects by interactions at peripheral opiate receptors [9]. Alternatively, the effects of dynorphin A (1-13) pretreatment on morphine bradycardia could be mediated at distinct anatomical locations (i.e., reflex centers within the central nervous system) [6] involved in the ultimate expression of morphine's bradycardic effects. Evidence that these interactions may not be mediated at a common anatomical site was obtained in preliminary studies. We observed that low doses of dynorphin A (1-13) injected centrally would also influence the peripherally-mediated bradycardic effects of IV morphine [9]. Therefore, an additional objective of these studies was to evaluate dynorphin A (1-13) interactions with intravenous morphine-induced bradycardia in unanesthetized rats and to establish the potential location(s) and mechanism(s) by which these interactions are mediated.

METHOD

Male Sprague-Dawley rats (200-300 g; Zivic Miller Labs) were given ad lib access to food and water, with lights on at 06:00 and off at 18:00 hours. On the day prior to pharmacological testing, rats were anesthetized and implanted with external jugular vein and tail artery catheters as previously described [18]. For rats receiving ICV injections, 30 ga stainless steel catheters were additionally implanted into the right lateral ventricle [18]. Rats were given free access to water, but were fasted overnight following surgery according to our standard protocol [8]. On the day following surgery, unanesthetized animals in their home cages were acclimated to the experimental environment. Heart rate and arterial pressure were continuously monitored using microtransducers (Narco-Bio Systems RP 1500i) connected to a Narcotrace 80 physiograph.

To define the short-term cardiovascular effects of dynorphin A (1-13) (Peninsula Labs), separate groups of animals were used for ICV, SC and IV studies. Dynorphin A (1-13)

was injected ICV (10 μ g (6.2 nmol) or 20 μ g (12.4 nmol)); control groups received equivolume saline (10 μ l or 20 μ l, respectively injected over 20-30 sec, followed by a 3 μ l flush). To evaluate the short-term effects of peripheral injections, rats received either IV or SC dynorphin A (1-13) (1 mg/kg (0.6 μ mol/kg)) or equivolume saline (1 ml/kg). These doses were chosen based upon prior reports [4, 9, 16], and in the case of ICV or IV administration, these were the minimal doses required for observable effects. Mean arterial pressure (MAP), pulse pressure (PP), and heart rate (HR) were monitored for one hour after injection.

To determine the effects of ICV dynorphin A (1-13) pretreatment on morphine bradycardia, rats were treated with dynorphin A (1-13) 10 μ g ICV or equivolume saline (10 μ l) at 10:00 hours. Four, six or eight hours later, morphine sulfate (0.1 mg/kg; Mallinckrodt) was injected IV. Since the bradycardic effects of these low doses of morphine are transient [9, 12, 13], cardiovascular responses were monitored for five minutes with less frequent sampling over the subsequent hour. Statistical comparisons between peak effects for individual experimental and control groups were made by the Student's *t*-test; ANOVA with repeated measures was used to assess interactions between time of treatment and the magnitude of bradycardic responses. Significance was assigned for $p < 0.05$.

RESULTS

Administration of dynorphin A (1-13) resulted in changes in cardiovascular function, both heart rate and mean arterial pressure (Fig. 1) as well as behavioral effects such as barrel rolling and wet dog shakes. Injection of dynorphin A (1-13) ICV at 10 μ g and 20 μ g doses produced a transient, dose-related increase in MAP (from a mean of 96 mmHg to 106 mmHg, and 94 mmHg to 116 mmHg, respectively) (Fig. 2). Conversely, IV administration of dynorphin A (1-13) resulted in a period of hypotension of a similar transient nature

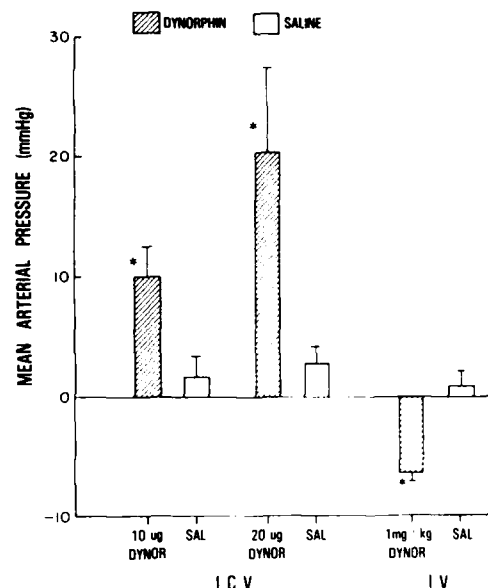


FIG. 2. Effects of dynorphin A (1-13) on mean arterial pressure in unanesthetized rats following intracerebroventricular (ICV) injections (10 or 20 μ g) or intravenous (IV) injection (1.0 mg/kg). Figure presents changes in MAP from baseline values. DYNOR represents dynorphin A (1-13) injected rats. SAL represents saline controls. Vertical bars are 1 SEM; $n=15-20$ rats/group; asterisks represent $p<0.05$.

(92 mmHg to 85 mmHg) (Fig. 2). Cardiovascular and behavioral responses ICV and IV dynorphin A (1-13) disappeared by five minutes post treatment. Subcutaneous dynorphin A (1-13) treatment had no effect on MAP at 1.0 mg/kg (data not shown). Heart rate was increased immediately post-injection in all experimental and control groups, probably due to handling of the animals during injection. There were no significant differences in HR responses between treated and control groups regardless of route of administration (data not shown).

Dynorphin A (1-13) ICV demonstrated a modulatory effect on intravenous morphine-induced bradycardia, with effects varying with the length of the interval (or time of day) between dynorphin A (1-13) and morphine treatment. With a four-hour pretreatment (animals tested at 14:00), dynorphin A (1-13) potentiated morphine bradycardia. Six hour dynorphin A (1-13) pretreatment (animals tested at 16:00) resulted in no significant difference from control values, while eight hour dynorphin A (1-13) pretreatment (animals tested at 18:00, immediately prior to lights off) significantly antagonized morphine bradycardia (Fig. 3).

Running counter to the trend seen in dynorphin A (1-13) treated groups, morphine alone exhibited increasing bradycardic efficacy later in the day as rats began their nocturnal activity cycle (Fig. 3). Using an ANOVA with repeated measures to analyze the bradycardic response 1 or 2 min after IV morphine, the saline pretreated group became progressively more bradycardic following morphine over time ($p<0.01$), whereas the dynorphin pretreated group demonstrated a progressive decrease in the bradycardic response to morphine over time ($p<0.01$). Further substantiating these different responses between groups over time, ANOVA revealed a significant interaction between saline and dynorphin A (1-13) pretreated groups ($p<0.005$). Thus,

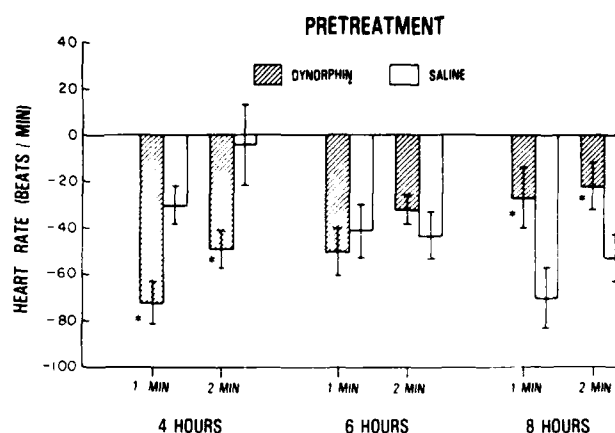


FIG. 3. Heart rate effects of IV morphine sulfate challenge (0.1 mg/kg) 4, 6, 8 hours after treatment with saline ICV (clear bars) or dynorphin A (1-13) (10 μ g ICV; hatched bars). Saline pretreated rats experienced a progressively greater degree of bradycardia as the day progressed from 14:00 (4 HOURS), to 16:00 (6 HOURS) to 18:00 (8 HOURS). By contrast, dynorphin A (1-13) ICV pretreatment resulted in a progressive decrease in the bradycardic actions of IV morphine sulfate over the time intervals shown (interactions: $p<0.05$; ANOVA; see text). Figure presents changes in heart rate from baseline values. Vertical bars represent 1 SEM; asterisks indicate significant differences within groups ($p<0.05$) at 1 MIN or 2 MIN. 1 MIN and 2 MIN represent time immediately following IV morphine sulfate injections.

as morphine bradycardia became progressively greater in control rats, dynorphin A (1-13) pretreated rats demonstrated progressively less of a bradycardic response to morphine.

DISCUSSION

The short-term cardiovascular effects of dynorphin A (1-13) were shown to vary with the route of administration. At the doses used, dynorphin's effects ranged from hypertension following ICV injection, no effect following SC administration and hypotension when injected IV. In a similar manner, intravenous injections of Substance P have been shown to produce hypotension, whereas the intracerebroventricular administration of Substance P elevates arterial pressure [16]. As emphasized previously [6], these data indicate that varying pharmacological effects of injected agonists can be evoked by different routes of administration. In addition, one recent report indicated that dynorphin A (1-13) produces bradycardia and hypotension in anesthetized rats [5], effects opposite those we observed in unanesthetized rats. However, as emphasized previously, anesthetics often reverse the direction of cardiovascular changes produced by opioid drugs [6,7]. Thus, due to the complexity of autonomic networks, pharmacological responses to injected agonists may have little or no relevance to their potential physiological role(s) in cardiovascular function.

Dynorphin A (1-13) was shown to have long lasting modulatory effects on IV morphine-induced bradycardia; these effects varied according to the time of dynorphin A (1-13) pretreatment relative to the time of day when morphine was injected. Four hour pretreatment with dynorphin A (1-13) was shown to potentiate morphine's effects (at 14:00), but with longer pretreatment intervals, this effect was diminished and ultimately reversed (at 18:00). Previous studies

have shown that anesthetized rats, subjected to arterial cannulation surgery immediately following dynorphin A (1-13) pretreatment, demonstrated a potentiation of morphine bradycardia when measured 30 minutes after SC dynorphin A (1-13) injection [12]. At two hours following SC dynorphin A (1-13) injection (1 mg/kg), those investigators observed a reversal of this effect, with dynorphin A (1-13) now antagonizing morphine bradycardia. The present studies, using ICV dynorphin A (1-13) pretreatment (10 μ g) with unanesthetized, unrestrained rats, corroborate those findings. However, in our studies, morphine injections in 4 hour dynorphin pretreated rats potentiated bradycardia, and 8 hour dynorphin pretreatment antagonized morphine bradycardia (Fig. 3). The differences between the time course of dynorphin A (1-13) actions of morphine bradycardia observed by Kiang and Wei [12] and those we obtained in these studies may be accounted for by different routes of injection, possible effects of the anesthesia, the time of surgical stress for catheter implantation or the time of day.

It has been suggested that morphine bradycardia is mediated at μ -receptors [6, 7, 11]. Furthermore, the opiate receptors which mediate the rapid bradycardic effects of IV morphine appear to be localized peripherally in the heart or major arteries (stretch or chemo receptors) or the lungs (pulmonary J-receptors) [12, 13]. It has also been proposed that dynorphin (and other κ agonists) can modulate the effects of μ or δ opioid agonist and antagonist ligands by interactions at a common macromolecular opioid receptor complex [10, 14, 15]. These present results demonstrating that central (ICV) injections of dynorphin A (1-13) can modulate peripheral effects of morphine suggest, however, that apparent interactions among opioid ligands may also occur by actions upon different anatomical sites connected via autonomic pathways. For example, dynorphin A (1-13) may act at higher

nervous centers involved with integrating cardiovascular function that, in turn, exert modulatory effects on the reflex responses evoked by peripheral morphine administration. Conversely, it remains possible that dynorphin A (1-13) injected ICV could enter the blood stream and act at a common peripheral receptor complex. However, the low dose of dynorphin A (1-13) used in central injections (10 μ g) relative to the effective IV dose (1 mg/kg) makes this an unlikely hypothesis. Although dynorphin A (1-13) is known to have actions upon kappa receptors, future studies are required to address whether the interactions we observed were mediated by specific opiate receptor subtypes.

Finally, the increasing magnitude of the bradycardic response to the same dose of IV morphine at different times of day indicates the importance of circadian variables in assessing cardiovascular responses to pharmacological substances. Similarly, it has been shown that the antinociceptive effects of morphine are also exaggerated as rats approach their nocturnal activity cycle [3]. These observations indicate that circadian rhythms could be responsible for the increase in morphine's bradycardic effects over time, and that the modulation of morphine bradycardia by dynorphin A (1-13) pretreatment is also affected by the time of day. It remains possible that dynorphin's modulatory effects could be exercised through an effect of this opioid peptide to disrupt circadian rhythms, resulting in an apparent potentiation or antagonism of morphine-induced bradycardia.

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