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Effects of Intrathecal Antagonists on the Antinociception, Hypotension, and Bradycardia Produced by Intravenous Administration of [D-Ala^2]-Methionine Enkephalinamide (DALA) in the Rat

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AICHER, S. A. AND A. RANDICH. *Effects of intrathecal antagonists on the antinociception, hypotension, and bradycardia produced by intravenous administration of [D-Ala^2]-methionine enkephalinamide (DALA) in the rat.* PHARMACOL BIOCHEM BEHAV **30**(1) 65–72, 1988.—DALA is a synthetic pentapeptide that produces inhibition of the tail-flick reflex evoked by radiant heat, as well as hypotension and bradycardia. Two experiments examined the effects of administration of various receptor antagonists into the subarachnoid space of the lumbar spinal cord on the antinociception produced by IV administration of DALA. Experiment 1 showed that intrathecal administration of 30 μg of phentolamine produced a significant reduction in the antinociceptive effect of DALA, while naloxone (30 μg), methysergide (30 μg), or vehicle control had no effect. Experiment 2 showed that intrathecal administration of combinations of either phentolamine and methysergide, or phentolamine and naloxone, were no more effective in reducing the antinociceptive effect of DALA than administration of phentolamine alone. These data demonstrate the involvement of descending noradrenergic systems in the production of antinociception by IV DALA. Further, they indicate that the antinociception produced by DALA is independent of a direct spinal action of the drug.

Intrathecal Antinociception Cardiovascular Pain DALA Vagus

SEVERAL studies have shown that intravenous (IV) administration of [D-Ala^2]-methionine enkephalinamide (DALA) produces inhibition of the tail-flick reflex to noxious heat, as well as, hypotension, bradycardia and apnea [10–13, 19, 20]. It has also been shown that these effects of DALA can be eliminated by bilateral cervical vagotomy [12,20]. Since DALA is a synthetic pentapeptide that does not readily cross the blood-brain barrier [8], activation of vagal afferents by DALA is at least a necessary condition for the effects noted above. Some of the medullary substrates necessary for the antinociceptive and cardiovascular effects of DALA have been delineated [10], but the nature of the spinal substrates is only partially understood. It has been shown that bilateral lesions of the dorsal portion of the lateral funiculi (DLFs) of the spinal cord eliminate the antinociceptive effect of DALA, but not the hypotensive effect [13]. However, the neurochemical substrates of systems originating in the medulla and descending in the DLFs to produce the antinociceptive action of DALA have not been determined. The purpose of the present study was to determine the neurotransmitter(s) involved in the inhibition of the spinally-mediated tail-flick reflex produced by IV administration of DALA. In this regard, norepinephrine, serotonin, and opioids have been consistently shown to mediate antinociception produced by activation of descending inhibitory systems [1, 3–7, 14, 18, 21]. Thus, receptor antagonists to these neurotransmitters were used in the present study.

METHOD

Subjects

Forty-two male Sprague-Dawley rats (350–475 g) 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, with food and water available on an ad lib basis.

Apparatus

Nociceptive responses were measured with a tail-flick apparatus. A radiant heat stimulus was provided by a projector bulb housed in a metal casing and focused on the rat's tail. Surface temperature of the tail increased from an ambient level of 38°C to a terminal level of 52°C over a ten second period during exposure to the heat stimulus with a 1.5°C per sec increase in temperature.

Arterial blood pressure and heart rate were recorded via the pulse pressure signal provided by a Century pressure

transducer, an Apple II+ computer, the FIRST system [16], and software developed by Marshall-Goodell and Randich. (The software program was developed by B. Marshall-Goodell with modifications by A. Randich. This program provides on-line recording of arterial blood pressure each second (100 samples/sec) with immediate conversions to true mean arterial blood pressure. Heart rate was triggered on the basis of pulse pressure with timing of inter-beat intervals and conversion to heart rate with an accuracy of approximately 2 bpm.)

Surgical Techniques

Each rat was anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). A catheter was implanted in the right jugular vein (Silastic) for drug administration and in the left common carotid artery (Microline) for recording of arterial blood pressure and a tracheostomy was performed. The rat was then placed in a stereotaxic device and the atlanto-occipital membrane was exposed and cut. A 7.5 cm intrathecal catheter (polyethylene-10) was inserted into the subarachnoid space of the spinal cord to the level of the lumbar enlargement for the administration of receptor antagonists [23]. The musculature was sutured over the injector end of the intrathecal catheter.

Testing

All testing was conducted on rats maintained in the lightly-anesthetized state by pentobarbital supplements of 0.05 ml (for details see [15]). Tail-flick trials were administered until latencies were 3–4 sec. Tail position was varied and the same position was not heated on any two consecutive trials, in order to prevent damage to the tail. The tail-flick latency was defined as the interval between the onset of heating and the withdrawal of the tail from the heat source, and was recorded automatically by the activation of a photocell. If a tail-flick reflex did not occur within 10 sec of heat onset, the heat stimulation automatically terminated. The baseline tail-flick trial used for purposes of data analysis was obtained 1 min prior to each IV drug administration. Test tail-flick trials occurred 0.25, 1, 2, 3, 4 and 5 min following IV injections. Arterial blood pressure and heart rate were monitored continuously.

All subjects received a pretest to IV DALA (500 μg/kg), followed by 0.20 ml saline flush, prior to intrathecal injection to verify that the drug would produce inhibition of the tail-flick reflex. A greater than 25% increase in the tail-flick index (TFI: see calculation below) at the 0.25- and 1-min test trials was set as the criterion for an inhibitory effect of DALA. Following the 5-min pretest to DALA, an intrathecal injection was given. Five min after the intrathecal administration, an IV injection of saline vehicle was given (volume equal to total volume for DALA injection) and testing performed for 5 min, at the same time points used for the DALA pretest. This saline test ensured that there were no effects of the IV vehicle itself, and that the animals could respond reliably following the intrathecal injection. Finally, 20 min after the initial intrathecal injection, a second IV DALA administration was given and 5 min of testing performed, to determine the effects of the intrathecal antagonist on the antinociceptive efficacy of DALA.

Experiment 1. Four groups of rats (N=6/group) received an intrathecal injection of either phentolamine, naloxone, methysergide, or saline. All antagonists were given in a 30 μg dose, in a volume of 7.5 μl followed by an 8 μl saline flush; for a total injectate volume of 15.5 μl. The total intrathecal injection time was 1 min. Control subjects received equal volume injections of saline alone. Following this injection, the effects of both saline IV and DALA IV on tail-flick latency, arterial blood pressure, and heart rate were assessed as described above.

Experiment 2. Three groups of rats (N=6/group) received intrathecal injection either of a combination of two antagonists, or saline vehicle. The phentolamine-methysergide group received sequential injections of 30 μg of each antagonist in 7.5 μl, followed by a single 8 μl flush, making a total injectate volume of 23 μl. The total intrathecal injection time was 3 min. A second group received sequential intrathecal injections of phentolamine and naloxone. The order of the phentolamine-methysergide and phentolamine-naloxone injections was counterbalanced within each group. The saline vehicle group received 23 μl of saline. Again, the effects of both saline IV and DALA IV on tail-flick latency, arterial blood pressure, and heart rate were tested following the intrathecal injection for each animal.

Histology

After testing was completed, the animal was sacrificed with an overdose of pentobarbital sodium. The placement of the intrathecal catheter was verified using an intrathecal injection of Fast Green dye (7.5 μl) followed by an 8 μl saline flush. The spinal cord was removed by hydraulic pressure [2] immediately after the dye injection and the location of staining on the cord was visualized. Only rats that had catheter placements that were both within 1.0 cm of the lumbar enlargement and did not penetrate the spinal cord were included in the experiment.

Data Analysis

Each tail-flick latency was converted to a tail-flick index (TFI) by the formula: [(test trial latency − baseline latency)/(10 sec − baseline latency) × 100]. A new baseline value was established before each IV injection.

Cardiovascular measures were recorded at the start of the 0.25-, 1-, 2-, 3-, 4- and 5-min trials to eliminate cardiovascular artifacts due to application of the heat stimulus. The largest change in cardiovascular function manifested prior to the 0.25-min trial was also recorded and defined as the "Peak" response. This response was recorded because of the transient nature of the cardiovascular changes produced by IV administration of DALA. Tail-flick indices (TFI), percentage change from baseline blood pressure, and percentage change from baseline heart rate (where peak responses were also included for cardiovascular measures) were subjected to an overall ANOVA. Where either significant main effects or interactions with the antagonist were obtained in the overall ANOVA, two-way ANOVAs (IV Drug × Intrathecal Antagonist) were performed at each test trial. When the two-way ANOVA was significant at a test trial, Newman-Keuls post hoc analyses were performed on the means, and it is the outcome of these analyses that is reported in the text. Pretest responses to DALA were analyzed in a similar fashion except that the follow-up analyses were one-way ANOVAs at each time point. Randomized block analyses were performed on the baseline cardiovascular measures over time (taken immediately prior to each of the 3 IV injections: DALA, saline and DALA) for each antagonist group to assess the effects of the intrathecal treatment on arterial blood pressure and heart rate. Finally, a Pearson's

TABLE 1

BASELINE VALUES FOR MEAN ARTERIAL BLOOD PRESSURE AND HEART RATE FOR GROUPS IN EXPERIMENTS 1 AND 2

Group	Arterial Blood Pressure (mmHg)			Heart Rate (BPM)		
	Baseline	5 min	20 min	Baseline	5 min	20 min
			Experiment 1			
Saline	105.0 ± 2.5	92.8 ± 0.5	90.0 ± 7.3	400.2 ± 11.9	383.5 ± 18.4	384.7 ± 19.1
Phentolamine	118.8 ± 6.8	99.7 ± 10.6	101.7 ± 7.6	424.2 ± 9.0	405.3 ± 12.1	410.5 ± 17.6
Naloxone	120.2 ± 10.1	118.7 ± 11.6	107.7 ± 9.7	426.3 ± 25.2	445.7 ± 26.1	429.2 ± 27.6
Methysergide	105.0 ± 9.5	88.8 ± 10.7	82.8 ± 5.5	365.2 ± 20.6	342.8 ± 23.6	354.3 ± 13.1
			Experiment 2			
Saline	123.0 ± 2.1	119.0 ± 5.5	118.3 ± 5.5	402.7 ± 16.7	409.7 ± 14.9	412.3 ± 14.0
Phentolamine-Methysergide	119.0 ± 7.9	90.3 ± 6.8	96.5 ± 9.8	407.0 ± 18.5	384.5 ± 22.3	395.7 ± 25.7
Phentolamine-Naloxone	119.8 ± 5.3	99.2 ± 5.0	112.8 ± 4.0	387.8 ± 13.2	388.2 ± 21.1	429.0 ± 13.0

Baseline measures were taken at three time points as follows: (1) prior to any drug treatment (Baseline); (2) 5 min after the intrathecal injection; (3) 20 min after the intrathecal injection of an antagonist or saline control.

Values shown are mean ± SEM, N=6 for each mean.

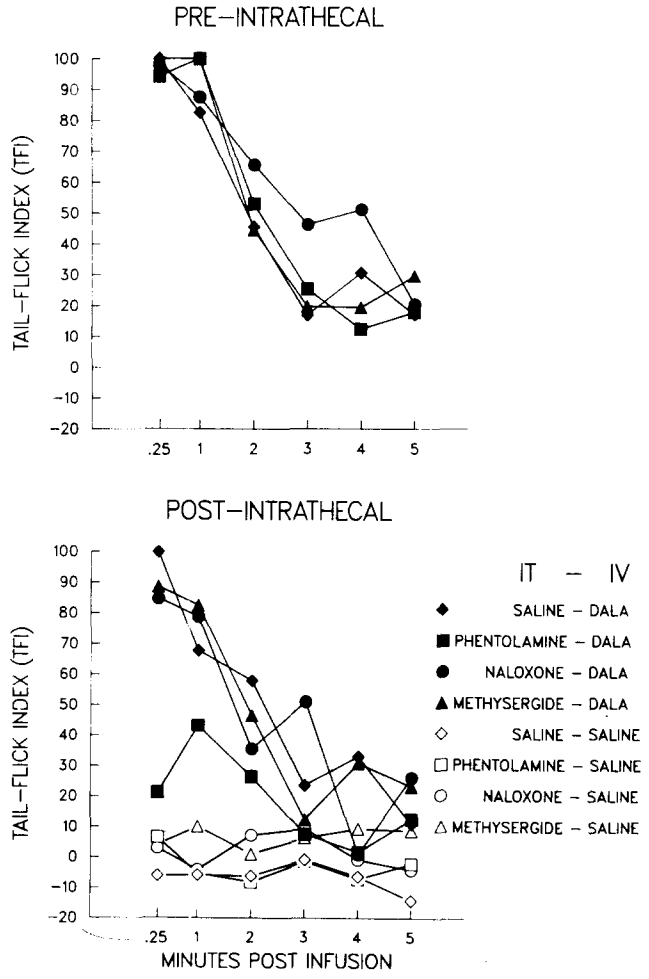

FIG. 1. Mean tail-flick index values for groups in Experiment 1 following IV DALA pretest (top panel) and following IV DALA (filled symbols) or IV saline (open symbols) after intrathecal injection (bottom panel) of saline (◇), phentolamine (□), naloxone (○), or methysergide (△), as a function of time.

correlation analysis was performed on the TFI values and either the blood pressure or heart rate response for all subjects in each experiment at the 0.25-min trial. Alpha was set at 0.05.

RESULTS

Experiment 1

The top panel of Fig. 1 shows the TFI values obtained during the pretest of DALA for the various groups as a function of time. DALA produced large elevations in tail-flick latency that dissipated over the 5-min testing period. An ANOVA indicated no significant between-groups differences during this pretest. The bottom panel of Fig. 1 shows the TFI values following IV administration of either saline or DALA in the presence of the intrathecal antagonist. In the saline-DALA, naloxone-DALA, and methysergide-DALA groups, the TFI values were significantly elevated compared to their TFI values following IV saline. However, the phentolamine-DALA group shows an attenuation of the antinociceptive effect. Newman-Keuls analyses of data derived from the overall ANOVA revealed that the mean TFI value for the phentolamine group receiving DALA was significantly lower than the TFI values of all other groups following DALA administration at the 0.25-min test trial. Moreover, the DALA-TFI value for the phentolamine group did not significantly differ from its own saline-TFI value at the 0.25-min test trial. After the 0.25-min test trial, statistical tests revealed no significant antagonist effects.

The top panel of Fig. 2 shows the depressor responses observed during the pretest of DALA, and an ANOVA of these data indicated there were no significant differences in the blood pressure response between groups. Table 1 presents baseline cardiovascular measures both prior to and following intrathecal drug administration. The values in the table indicate that for all groups there was some decrease in arterial blood pressure throughout the experiment. However, randomized block analyses of these values for each antagonist group revealed that the change in blood pressure was only significant in the phentolamine and naloxone groups. The bottom panel of Fig. 2 shows the blood pressure responses evoked by either IV saline or DALA in the pres-

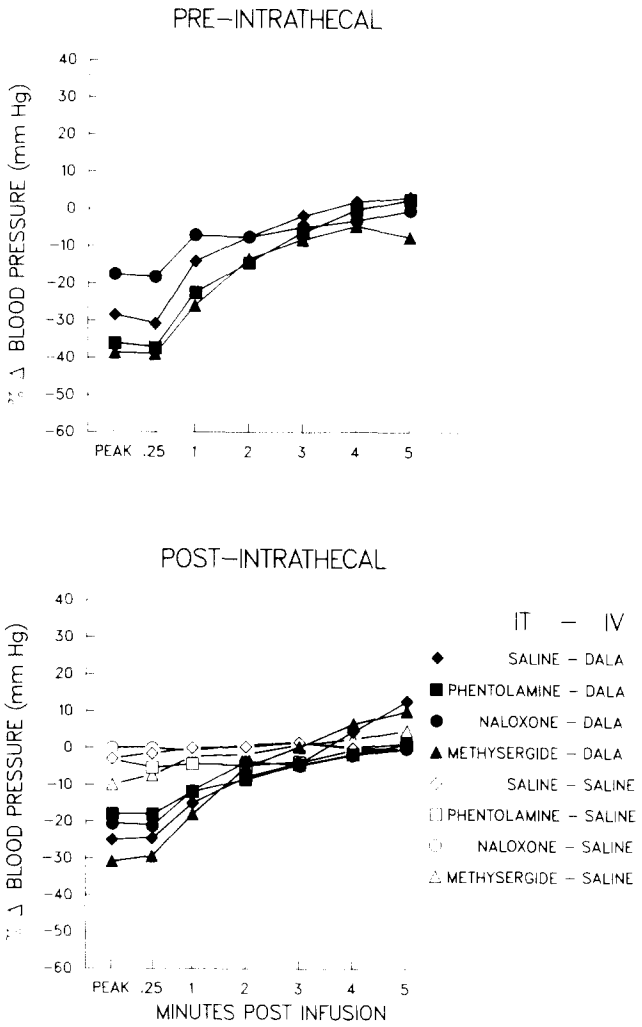

FIG. 2. Mean percentage change in arterial blood pressure for groups in Experiment 1 following IV DALA pretest (top panel) and following IV DALA (filled symbols) or IV saline (open symbols) after intrathecal injection (bottom panel) of saline (◇), phentolamine (□), naloxone (○), or methysergide (△), as a function of time.

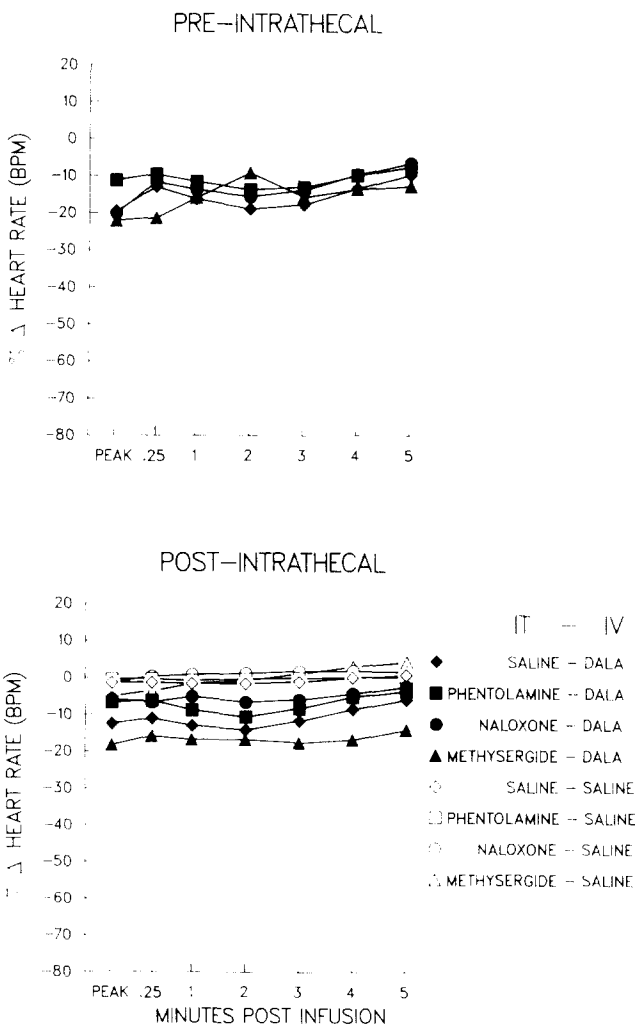

FIG. 3. Mean percentage change in heart rate for groups in Experiment 1 following IV DALA pretest (top panel) and following IV DALA (filled symbols) or IV saline (open symbols) after intrathecal injection (bottom panel) of saline (◇), phentolamine (□), naloxone (○), or methysergide (△), as a function of time.

ence of the intrathecal antagonist. Again, DALA produced depressor responses in all groups, and there was no significant antagonist effect in the overall ANOVA.

The top panel of Fig. 3 presents the heart rate responses obtained during the pretest of DALA, indicating a small and sustained bradycardic response to DALA administered IV. An ANOVA of these data indicated no significant between-groups differences. Table 1 shows the mean baseline heart rate values obtained both prior to and following intrathecal antagonist administration. The data indicate that heart rate remains fairly stable over time in the presence of the intrathecal antagonists, but that in general the methysergide group had a lower absolute heart rate than the other groups. The bottom panel of Fig. 3 shows that the IV administration of DALA in the presence of the intrathecal receptor antagonists continues to evoke a bradycardic response, whereas IV saline had virtually no effect on heart rate. The overall ANOVA of these data indicated a significant effect of antagonist, $F(3,20)=4.27$, but no interaction with Drug or Time. Post hoc analyses of the means collapsed across time revealed that the methysergide group showed significantly greater bradycardia than the other groups.

Experiment 2

Figure 4 presents the TFI values for groups receiving combined intrathecal injections. The top panel shows that all groups showed antinociception following IV DALA in this pretest and there were no significant between-group differences. The bottom panel shows that the IV injection of saline alone in the presence of the receptor antagonists did not increase TFI values, and also that the intrathecal injection of saline vehicle had no effect on the efficacy of IV DALA to produce antinociception. However, the administration of either phentolamine and methysergide, or phentolamine and naloxone, resulted in a significant reduction of the antinociception produced by DALA, $F(1,15)=17.66$. These groups differed significantly from the saline control group administered IV DALA at both the 0.25- and 1-min test trials, but did not differ from each other. Further, the mean TFI values for both groups at the 0.25-min test trial did not

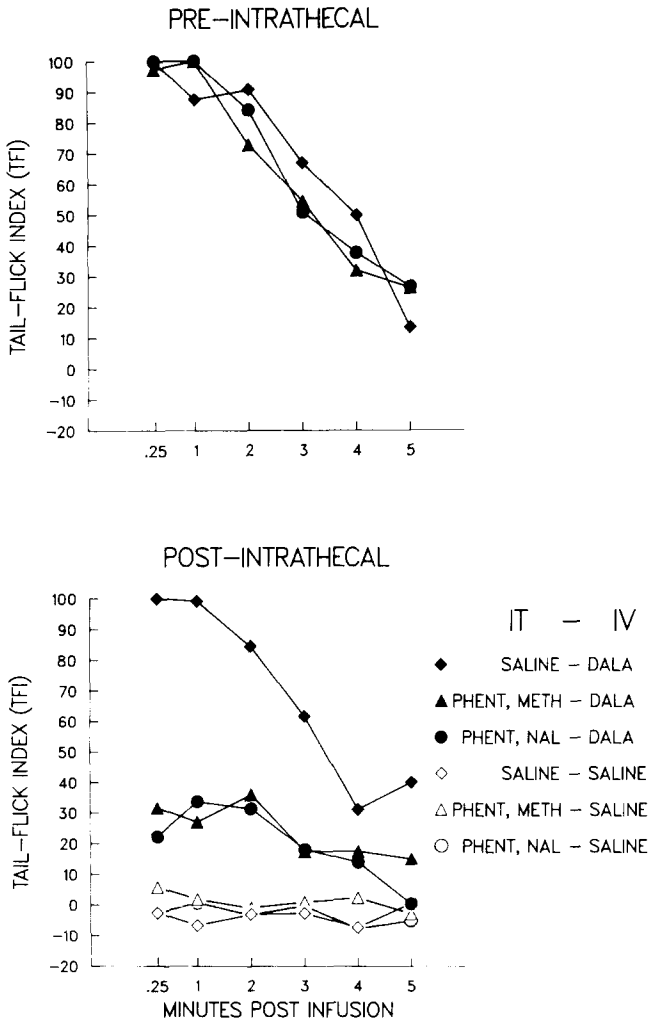

FIG. 4. Mean tail-flick index for groups in Experiment 2 following IV DALA pretest (top panel) and following IV DALA (filled symbols) or IV saline (open symbols) after intrathecal injection (bottom panel) of saline (◇), phentolamine and methysergide (△), or phentolamine and naloxone (○), as a function of time.

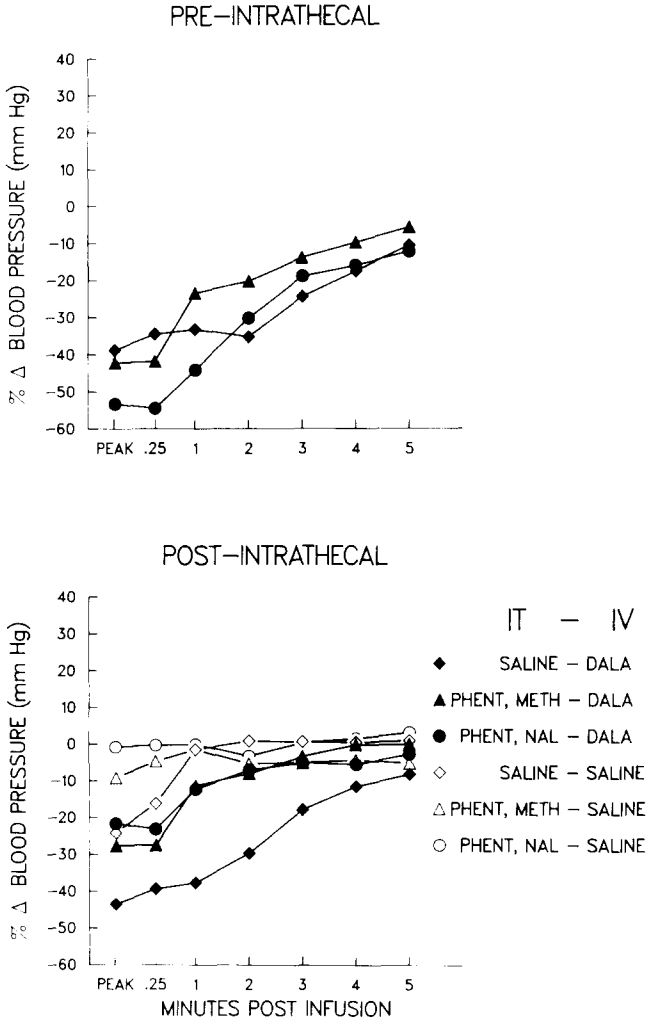

FIG. 5. Mean percentage change in arterial blood pressure for groups in Experiment 2 following IV DALA pretest (top panel) and following IV DALA (filled symbols) or IV saline (open symbols) after intrathecal injection (bottom panel) of saline (◇), phentolamine and methysergide (△), or phentolamine and naloxone (○), as a function of time.

differ from the values at the same time point following IV saline. At the 1-min test trial, the mean TFI value for the phentolamine-naloxone group was significantly less than the intrathecal saline group, but was also significantly greater than its own IV saline-TFI value. At the 1-min test trial the phentolamine-methysergide group showed a mean TFI value following IV DALA that did not differ from its own IV saline-TFI value. The mean TFI values for the two antagonist groups were not significantly different from each other at the 1-min test trial. No significant effects of the antagonists were found after the 1-min test trial.

The top panel of Fig. 5 shows that all groups showed a large depressor response to DALA during the pretest. An ANOVA of these data indicated that there was a significant difference in the magnitude of this pretest response for the groups as a function of time, $F(12,90)=2.21$. However, one-way ANOVAs failed to reveal any differences between groups at any single time value. Table 1 presents the baseline blood pressure values both prior to and following the intrathecal treatments. There was a decrease in arterial blood pressure over time in all groups, and this decline was statistically significant in both the phentolamine-methysergide and the phentolamine-naloxone groups. The lower panel of Fig. 5 presents the changes in arterial blood pressure following IV administration of saline and DALA in the presence of the combined intrathecal antagonists or intrathecal saline vehicle. The overall ANOVA of these data revealed a significant effect of antagonist, $F(2,15)=5.75$. The depressor response following DALA administration was significantly reduced at the 1- and 2-min test trials in the phentolamine-methysergide and phentolamine-naloxone groups, as compared to the group that received intrathecal saline, and also at the 3-min test trial for the phentolamine-naloxone group. The DALA means for the phentolamine-methysergide and phentolamine-naloxone groups also differed from their own IV saline means through the 1-min test trial.

The top panel of Fig. 6 shows that IV administration of DALA caused a reliable and sustained bradycardia during

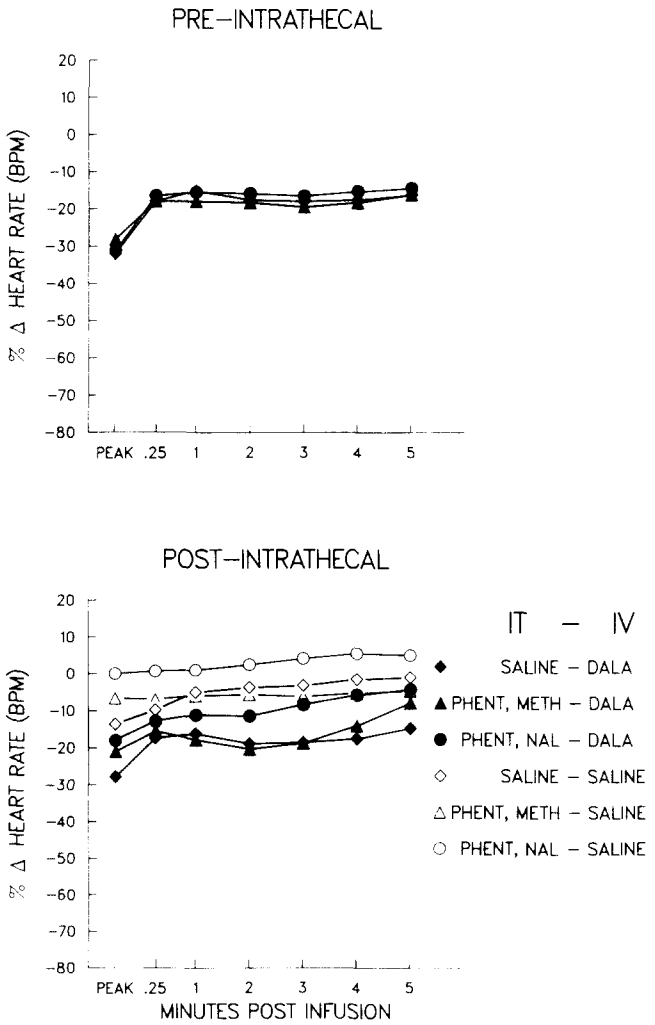

FIG. 6. Mean percentage change in heart rate for groups in Experiment 2 following IV DALA pretest (top panel) and following IV DALA (filled symbols) or IV saline (open symbols) after intrathecal injection (bottom panel) of saline (◇), phentolamine and methysergide (△), or phentolamine and naloxone (○), as a function of time.

the pretest and an ANOVA of these data revealed no significant between-groups differences. Table 1 presents the baseline heart rate measures both prior to and following the administration of the intrathecal antagonists. There were no significant changes in baseline heart rate across time for any of the antagonist groups. The bottom panel of Fig. 6 shows that DALA still produced a bradycardic response following the intrathecal injections. The overall ANOVA of these data indicated a significant main effect of antagonist, $F(2,15)=9.41$, but no interaction with Drug or Time. The values were collapsed across time for comparisons and Newman-Keuls analyses of the collapsed means following IV DALA showed that the phentolamine-naloxone and saline values differed significantly; these were the smallest and largest values respectively. Also, the response of the phentolamine-naloxone group to IV saline collapsed across time differed significantly from those of the other two groups following IV saline. These results indicate that the bradycardic responses to both IV saline and IV DALA were attenuated in the phentolamine-naloxone group.

DISCUSSION

The first experiment demonstrated a noradrenergic/adrenergic component in the inhibition of the tail-flick reflex resulting from IV administration of DALA, since intrathecal administration of phentolamine significantly reduced the antinociception produced by DALA. No significant attenuation of the antinociceptive effect of DALA was observed with intrathecal administration of either naloxone or methysergide, when compared to the saline control group. It is possible that any effect of these antagonists could have gone undetected if the dose was too small. However, this account is unlikely to be correct, since we have routinely observed that these doses of naloxone and methysergide are clearly suprathreshold for blocking descending inhibitory effects produced by activation of various CNS structures that result in either comparable or greater inhibition of the tail-flick reflex. The ability to block descending inhibitory systems with these and smaller doses of methysergide or naloxone has also been reported routinely by others [1, 4–7, 14, 18, 21]. These doses of methysergide and naloxone are also more than adequate to attenuate the inhibitory effects of the application of their respective agonists directly to the intrathecal space of the lumbar spinal cord [17,24]. Specifically, doses of methysergide of approximately 4–17 μg caused dose-dependent attenuation of the inhibition of the tail-flick reflex produced by the application of 200 μg of 5-HT to the lumbar cord [17]. Intrathecal naloxone (0.1–3.0 μg) caused dose-dependent attenuation of the effects of a variety of narcotic analgesics applied to the spinal cord, including morphine in doses of approximately 1.0–30 μg [24]. The highest doses of morphine in this study were sufficient to produce levels of antinociception comparable to those seen in the present study, i.e., 100% maximal effect with a 10 sec cut-off latency for the tail-flick response.

Experiment 2 examined combinations of receptor antagonists, since most forms of descending inhibition have been shown to have multiple medullary and spinal substrates [1, 4–7, 14, 18, 21], and would require the administration of combinations of intrathecal antagonists to generate complete blockade of descending inhibitory effects. The present experiments failed to reveal any further blockade of the inhibitory effects of DALA when phentolamine was administered in combination with either naloxone or methysergide. At most time points in both experiments, the groups that received phentolamine (alone or in combination with other antagonists) showed mean TFI values following DALA administration that did not statistically differ from their TFI values following IV saline. Therefore, it is possible that the inhibition produced by DALA is solely mediated by a (nor)adrenergic system. Graphically, however, the values for the phentolamine group given DALA appear to be greater than their saline control values. It is possible that a second transmitter system (descending or intrinsic to the spinal cord) is being activated by IV administration of DALA to contribute to the inhibitory effects seen in the presence of phentolamine. Alternatively, the failure to achieve complete blockade at all time points may be due to insufficient potency of the antagonist. If this interpretation is correct, it might be expected that administration of higher doses of phentolamine would result in complete blockade of the antinociceptive effect of IV DALA.

The effectiveness of phentolamine and the ineffectiveness of methysergide when administered either alone or in combination with phentolamine stands in contrast to a large

number of studies demonstrating that both noradrenergic/adrenergic and serotonergic systems mediate descending inhibitory effects produced by either electrical or chemical stimulation of lateral reticular nucleus [5], nucleus raphe magnus [1,4], and periaqueductal gray [1, 6, 7, 21], as well as other manipulations that produce antinociception [14,18]. On the other hand, the failure to find any effect of serotonergic receptor blockade is consistent with a recent lidocaine-mapping study [10] of medullary sites supporting DALA-induced antinociception. This study found no evidence for the involvement of the NRM-MRF regions, a primary source of descending serotonergic fibers, in DALA-induced antinociception. However, it is not yet completely understood how the activation of vagal afferents by DALA may interact with various brainstem regions that have been implicated in descending spinal inhibition through the use of direct chemical or electrical stimulation.

In Experiment 1, intrathecal administration of phentolamine attenuated the antinociception produced by IV administration of DALA with no significant effect on the cardiovascular responses. However, in the second experiment there did appear to be a greater attenuation of the depressor response to DALA following the administration of the intrathecal antagonists. These data would seem to imply that there may be a direct action of the antagonists on the cardiovascular responses to DALA. On the other hand, these results must be interpreted with caution because there were differences in the magnitude of the cardiovascular responses between the two experiments (compare Figs. 2 and 5 for example), as well as some between-group differences in pretest cardiovascular responses to DALA (blood pressure in Experiment 2). In order to address the issue of whether a relationship exists between the magnitude of the antinociception and the cardiovascular responses to the drug, correlation analyses (Pearson's r) were conducted on TFI values and percentage change measures using either blood pressure or heart rate values obtained at the 0.25-min trial following IV DALA administration for all subjects regardless of intrathecal treatment. The analyses on TFI and blood pressure revealed that the percentage change in arterial blood pressure evoked by DALA was significantly correlated with the degree of antinociception at the 0.25-min test trial for both Experiment 1 ($r=-0.530$) and Experiment 2 ($r=-0.683$). The percentage change in heart rate also was significantly correlated to the TFI values at the 0.25-min test trial in Experiment 1 ($r=-0.440$), but this correlation was not significant in Experiment 2 ($r=-0.399$). These results indicate that although there were no consistent between-group differences in the cardiovascular responses following DALA, the magnitude of the cardiovascular changes were significantly related to the magnitude of the inhibition of the tail-flick reflex evoked by DALA. However, it is important to note that all groups receiving intrathecal phentolamine (alone or in a combination) showed a significant decrease in baseline arterial blood pressure during the experiment (Table 1). Therefore, it is possible that the magnitude of the depressor response in all of the phentolamine groups was attenuated following IV DALA simply because they already had a lower basal blood pressure.

These data indicate that the cardiovascular and antinociceptive effects of IV DALA administration are not consistently separable from one another. It is reasonable to assume that the CNS substrates evoking these peripheral hemodynamic and respiratory effects are coupled with the effector component of the somatosensory effects, thereby resulting in at least a correlative relationship between the cardiovascular and antinociceptive effects of the drug. However, other data indicate that the peripheral hemodynamic and respiratory changes evoked by activation of the vagus do not directly contribute to the observed inhibitory effects [9,14].

Previous studies have shown that DALA can produce antinociception when applied directly to the spinal cord, and this antinociception can be antagonized by systemic administration of naloxone [22]. However, the present data support the view that DALA exerts its antinociceptive effect by vagally-induced activation of descending inhibitory systems of the medulla [10–13] because naloxone was ineffective in altering the antinociception when directly applied to the lumbar spinal cord. Therefore, there is no significant contribution of a direct spinal action of DALA to the antinociceptive effects seen following IV administration of the drug.

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