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Supraspinal Cerebral Areas Involved in Morphine's Intestinal Inhibition and Analgesia

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PAROLARO, D., M. SALA, G. PATRINI, P. MASSI, G. GIAGNONI AND E. GORI. *Supraspinal cerebral areas involved in morphine's intestinal inhibition and analgesia.* PHARMACOL BIOCHEM BEHAV 30(2) 319–324, 1988.—To explore the neuroanatomical pathways involved in mediating the antipropulsive effect and analgesia of morphine (M) in the periaqueductal gray matter (PAG), we examined the influence of the vagus nerve and the role of serotonergic neurotransmission. M-induced inhibition of intestinal transit was unaffected by subdiaphragmatic vagotomy. In contrast, electrolytic lesions in the raphe magnus nucleus (NRM) and pretreatment with a selective neurotoxin (5,6-DHT, 15 μg/rat) in the same region, both significantly reduced M-induced inhibition of intestinal transit. Analgesia was only slightly affected. p-CPA pretreatment (100 mg/kg IP) induced the same results. Finally some other central brain regions were found to be sensitive to M's intestinal inhibition and analgesia such as the mid-line thalamus, the dorsal and lateral hypothalamus, and the bulbar reticular formation. Negative results were obtained for frontal cortex, caudate and amygdala. Some considerations are put forward about the existence in the central nervous system of selective areas involved in intestinal modulation and their relation with those mediating pain transmission.

Morphine Intestinal effect Analgesia Central brain areas

EVIDENCE for central opioid-sensitive sites of inhibition of gastrointestinal transit comes primarily from animal studies in which morphine-like drugs or endogenous peptides were administered directly into the central nervous system (CNS). Observations in mice [9,17], rats [5, 8, 11], guinea pigs [17] and cats [20] indicate that morphine (M) applied intracranially by several techniques produces centrally initiated intestinal inhibition. Still, very little is known of the precise cerebral sites involved in intestinal reponses.

We recently demonstrated [16] the importance of the midbrain periaqueductal gray matter (PAG) in mediating M's intestinal effect. M is more active when injected into the PAG than into the cerebral ventricles (ICV) and single and bilateral lesions into the PAG reduced the antipropulsive effect of M given ICV. We also found that within the PAG the intestinal effect of M generally overlapped with the analgesic effect and appeared to be mediated mainly by μ receptors [12, 14]. In view of these findings, the first aim of the present work was to explore thoroughly the neuroanatomical pathways involved in mediating the antipropulsive effect of M in the PAG. We specifically examined the influence of the vagus nerve and the role of serotonergic neurotransmission both on the antipropulsive effect and on analgesia.

M was also microinjected into some other central structures known to be involved in mediating M analgesia, to evaluate gastrointestinal inhibition.

METHOD

Animals

Male Sprague-Dawley rats weighing 280–320 g were fed a pellet diet with water ad lib. Environmental conditions were standardized (22±2°C, 60% humidity and 12 hr artificial lighting per day). Before treatment the animals were randomized according to a complete block design and fasted for 15–18 hr.

Microinjections Into the Central Nervous System (CNS)

Microinjections into the various central structures were made as previously described [12] through 33-gauge stainless steel cannulae inserted into permanent guides extending 1 mm below their tips into the intended sites (Table 1).

Electrolytic Lesions

Electrolytic lesions were made in the raphe magnus nucleus (NRM), determined stereotaxically (AP −9.8; L 0; V 9.25 from bregma) by passing a 2 mA DC anodal current for 30 sec through a stainless steel insect pin (No. 00) insulated except for approximately 0.5 mm at the tip. A rectal cathode was used.

5-HT Depletion

One group of rats was microinjected with 5,6-dihydroxy-

TABLE 1

THE STEREOTAXIC COORDINATES FOR EACH CENTRAL SITE WERE CHOSEN ACCORDING TO THE ATLAS OF PAXINOS AND WATSON [15]

	AP (mm from bregma)	L	V
Frontal cortex	−0.3	2.5–3	1.5–1.8
Caudate	−0.3	3.5	6
Amygdala	−3.8	3.5	9.5
Dorsal thalamus	−2.8	2	4.7
Mid-line thalamus	−2.3	0	6.0–7.5
Ventral thalamus	−3.3	2.5	6
Dorsal hypothalamus	−2.8	0.5	8.3
Ventromedial hypothalamus	−2.3	0.7	9.5
Lateral hypothalamus	−2.8	2	9
Bulbar reticular formation	−11.8	0.7–1.8	9.5–10.5

tryptamine (DHT) (15 μg/rat) into the NRM tested 10 days later. A second group received 100 mg/kg IP of DL-p-chlorophenylalanine (p-CPA) methyl ester for three days; 24 hr after the last p-CPA injection rats received M (μg/rat) into the PAG.

5-HT was determined in spinal cord according to the method of Lackovic [7].

Histology

To verify the placement of cannulae and electrodes, the brains were removed and put in 10% formalin; 24 hr later all the brain blocks were frozen, cut to a thickness of 60 μm and alternate sections were mounted and stained with buffered safranin according to Wolf and Yen [22].

Vagotomy

Subdiaphragmatic vagotomy was performed according to the method of Martin *et al.* [10]. Briefly, the left gastric artery was isolated, ligated and cut.

The esophagus extending below the diaphragm was scraped free of connective tissue using a scalpel blade and then rubbed with 70% ethanol. Obvious connective tissue was also removed from the stomach. Animals undergoing sham vagotomy had their esophagi manipulated without the removal of connective tissue or severing of the left gastric artery and no ethanol was applied. Animals that had been vagotomized had greatly distended stomachs.

Intestinal Transit Assay

Intestinal transit was assessed 40 min after treatment with M on the basis of the progression of a charcoal meal through the small intestine as reported elsewhere [11]. The results were expressed as percentage of inhibition versus control transit (T_c) as follows:

$$(T_c - T_t)/T_c \cdot 100$$

where T_t was the transit in treated animals.

Analgesic Assay

Antinociceptive activity was assessed at 40 min in the same rats using a tail-flick method [3]. The results were expressed as percentages of the maximum possible effect (M.P.E.), calculated as follows:

$$\%\text{M.P.E.} = \frac{(\text{drug latency} - \text{control latency})}{15 - \text{control latency}} \cdot 100$$

where drug latency was the response time in seconds of drug-treated animals at 40 min and control latency was the pre-drug response time for each rat. A maximum cut-off of 15 sec was used and the rat was removed from the tail-flick apparatus if it failed to respond in this time.

Drugs

The following drugs were used: morphine hydrochloride (S.I.F.A.C., Milan, Italy), 5,6-dihydroxytryptamine (DHT) (Sigma Chemical Co., St. Louis, MO), DL-p-chlorophenylalanine methylester (Sigma Chemical Co., St. Louis, MO), all dissolved in saline.

For central microinjections the volume was limited to 1 μl to minimize diffusion and tissue damage.

Statistical Analysis

One-way analysis of variance (ANOVA) was performed by collapsing data across all groups. This analysis was followed by individual group comparisons with Tukey's W procedure or Student's *t*-test [19].

RESULTS

Vagotomy itself did not affect the intestinal action of 10 μg of M into the PAG and in fact the block of gastrointestinal propulsion produced by the opioid was not reduced in vagotomized rats. Vagotomized rats developed full analgesia (Fig. 1).

Several kinds of procedures were employed to ascertain the role of the serotonergic system in mediating the gastrointestinal inhibition evoked by M into the PAG. The typical NRM electrolytic lesion destroyed a considerable portion of this region (Fig. 2). Sham-lesioned rats presented typical gastrointestinal propulsion after M microinjections into the PAG but the effect of M in lesioned rats was significantly attenuated (Tukey's W sham + M vs. lesioned + M = 92.31, $p<0.01$). Lesions in this area also slightly reduced M's analgesic effect (about 20%).

Pretreatment with a serotonergic neurotoxin, 5,6-DHT, into the NRM 10 days before M into the PAG, significantly influenced M's gastrointestinal inhibition (from 70% to 16% in lesioned rats) (Fig. 3). 5,6-DHT caused a significant reduction in spinal cord 5-HT concentration.

Moreover, in 5,6-DHT-pretreated rats, the analgesic effect was slightly diminished. The selective depletion of 5-HT by p-CPA pretreatment also significantly reduced M's antipropulsive effect (Tukey's W Saline + M vs. p-CPA + M = 60.18, $p<0.01$), and resulted in a small reduction of analgesia (Fig. 4).

Finally the effects of microinjections of 10 μg/rat of M in various supraspinal brain centers on gastrointestinal inhibition and analgesia are illustrated in Figs. 5, 6 and 7. Microinjections into the frontal cortex, caudate or amygdala did not induce intestinal inhibition (Fig. 5). A slight analgesic effect was observed only in the frontal cortex.

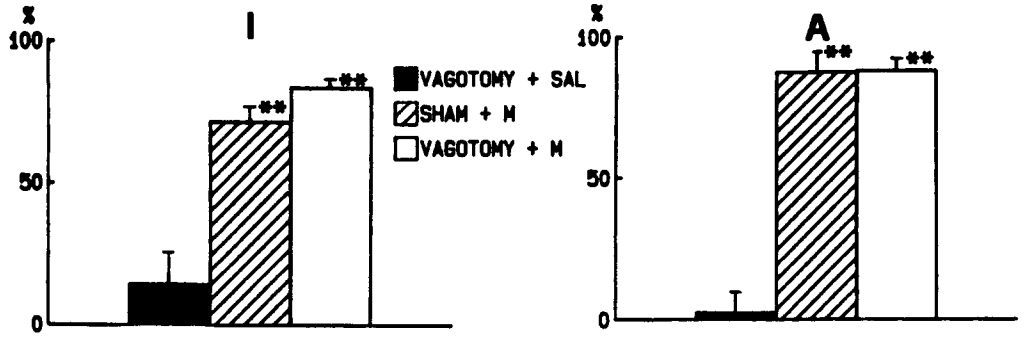

FIG. 1. Effect of vagotomy on intestinal inhibition (I) and analgesia (A) after microinjections of M (10 μg/rat) into the PAG. Ordinate: percentage of intestinal transit inhibition on the left and percentage of the maximum possible analgesic effect on the right. Bars represent mean±SEM responses of six animals. $^{**}p<0.01$ (Tukey's test) vs. vagotomy + saline group.

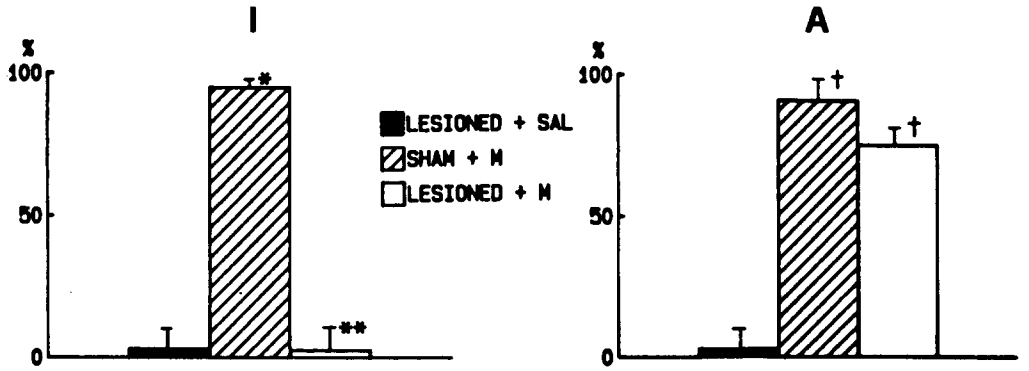

FIG. 2. Effect of electrolytic lesions into the raphe magnus nucleus (NRM) on intestinal inhibition (I) and analgesia (A) after microinjections of M (10 μg/rat) into the PAG. Bars represent mean±SEM responses of five animals. I: $^{*}p<0.01$ vs. lesioned + saline group; $^{**}p<0.01$ vs. sham lesioned + M group; A: $\dagger p<0.01$ vs. lesioned + saline group.

FIG. 3. Effect of 5,6-DHT pretreatment on intestinal inhibition (I) and analgesia (A) after microinjections of M (10 μg/rat) into the PAG. Bars represent mean±SEM responses of five rats. I: $^{*}p<0.05$ vs. sham lesioned + M group; $^{**}p<0.01$ vs. lesioned + saline group; A: $\dagger p<0.01$ vs. lesioned + saline group.

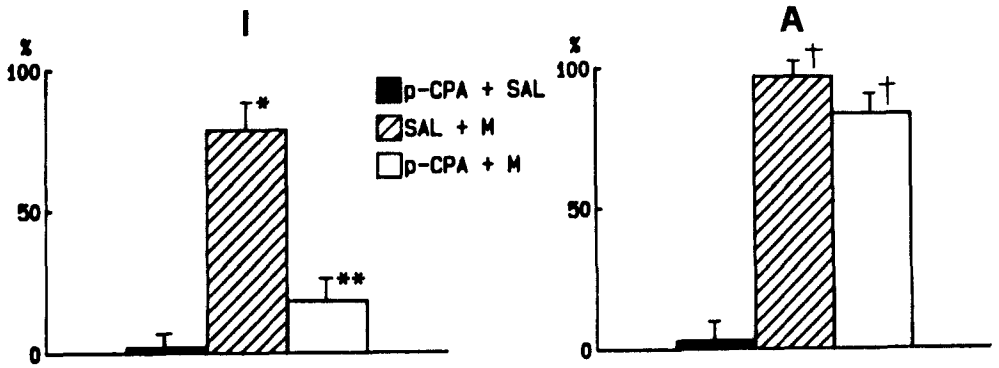

FIG. 4. Effect of p-CPA pretreatment on intestinal inhibition (I) and analgesia (A) after microinjections of M (10 μg/rat) into the PAG. Bars represent mean±SEM responses of five animals. I: $*p<0.01$ vs. p-CPA + saline group; $**p<0.01$ vs. saline + M, group; A: $\dagger p<0.01$ vs. p-CPA + saline group.

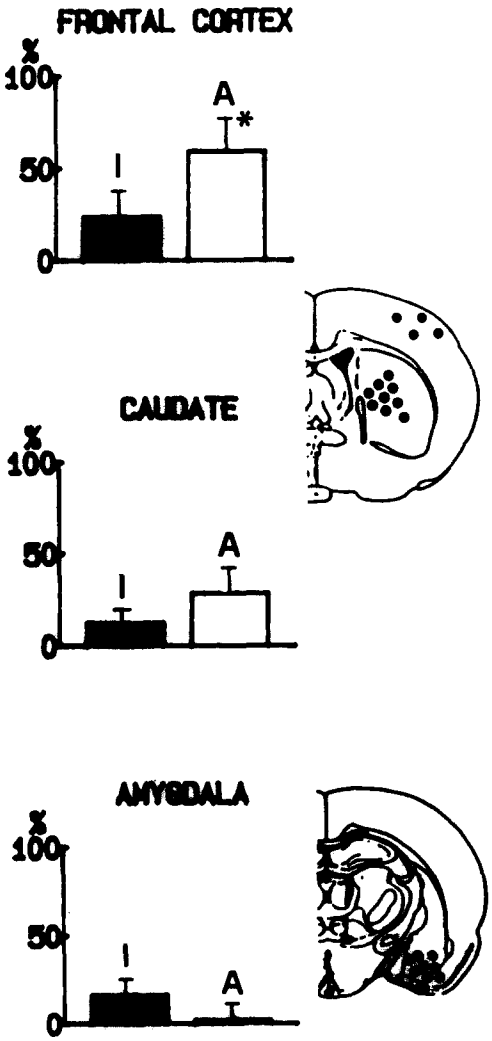

FIG. 5. Cannula mapping study of intestinal inhibition (I) and analgesia (A) induced by microinjections of M (10 μg/rat) in three CNS regions, frontal cortex, caudate and amygadala. Results of histological analyses are illustrated to the right on coronal drawings of the rat brain taken from Paxinos [15]. $*p<0.05$ vs. controls.

When the brain cannulae were placed in the thalamus or hypothalamus some interesting data were found. First, in the thalamus only the nuclei of the mid-line were sensitive to M (intestinal inhibition and analgesia were about 100%); results were negative for both these effects in the dorsal or ventral areas (Fig. 6).

Within the hypothalamus, there was a significant inhibition of intestinal propulsion coupled with analgesic effect in the lateral and dorsal regions. The ventromedial area was ineffective for intestinal effect though moderate analgesia was elicited (Fig. 6). Finally M microinjected into the bulbar reticular formation (BRF) markedly evoked both the antitransit and analgesic effects (Fig. 7).

DISCUSSION

Our results support the concept that several brain areas may be involved in mediating M-induced intestinal inhibition. This study provides evidence of the peripheral efferent pathway, activated by M microinjected into the PAG, which is involved in the gastrointestinal effect.

The antipropulsive action of M was not abolished by subdiaphragmatic vagotomy, thus excluding the influence of the vagus nerve. These data closely agree with our findings with dermorphin [13] but conflict with those of Stewart *et al.* [20] according to whom vagotomy partly abolished the intestinal effect of ICV M.

The serotonergic system appears to play an important role in mediating the antitransit effect of M into the PAG. When the integrity of the 5-HT pathway was affected by 5,6-DHT or p-CPA pretreatment, M's intestinal inhibition was strongly reduced.

Analgesia was less affected by these treatments. This is surprising since it is widely accepted that analgesia produced by centrally acting M is mainly due to the interaction with descending spinal serotonergic neurons which originate from raphe nuclei [1,4]. However it is now becoming increasingly evident that the raphe-spinal system is more complex than had been previously thought and contains both monoaminergic (noradrenaline, dopamine) and peptidergic (neurotensin, CCK, substance P) neurons [2,4]. So it can be hypothesized that the high degree of analgesia still present in rats with a damaged 5-HT system may be ascribed to an interaction with other neurotransmitters. This seems reason-

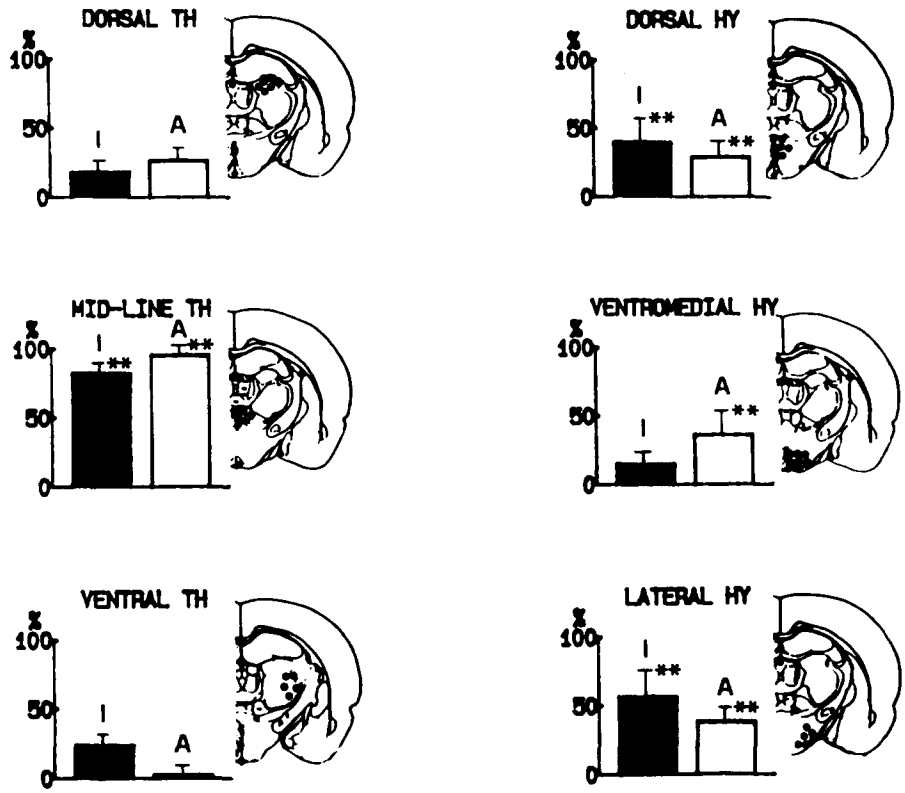

FIG. 6. Cannula mapping study of intestinal inhibition (I) and analgesia (A) induced by thalamic and hypothalamic microinjections of M (10 μg/rat). Three thalamic areas, dorsal, mid-line, and ventral, and three hypothalamic areas, dorsal, ventromedial and lateral, were examined. $^{**}p<0.01$ vs. controls.

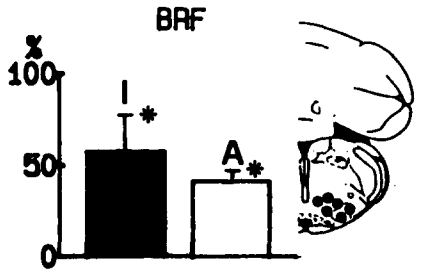

FIG. 7. Cannula mapping study of intestinal inhibition (I) and analgesia (A) induced by microinjections of M (10 μg/rat) into the bulbar reticular formation (BRF). $^{*}p<0.01$ vs. controls.

able considering that the dose of M we employed to elicit intestinal inhibition (10 μg/rat) is much larger than that causing analgesia, so other neuromediators could well have been influenced. Some general conclusions can be drawn from the findings concerning the involvement of other brain regions in mediating M's gastrointestinal effect and analgesia.

First, the centers mediating M intestinal inhibition cannot be separated from that mediating analgesia. In all tested sites in a closely related manner, M regularly elicits analgesia and decreased intestinal transit. The areas found sensitive to M's antitransit effects belong to the pain transmission system, possibly indicating that the same CNS centers control both visceral function and the analgesia elicited by the opiods. In the future a variety of pharmacological and functional approaches could be taken to help establish whether the central visceral control of opioids can be attributed—as already widely accepted for analgesia[1]—to activation of a descending system which influences the gut circuitry probably through several neurotransmitters.

Finally, several explanations can be offered for the negative results of tests in some regions.

The lack of central gastrointestinal effect found in frontal cortex, caudate and amygdala could be attributed to a low density of opioid receptors in the cortex and to mainly delta receptors in the other two areas [18]. This type of opioid receptor is in fact known not to be involved in gastrointestinal inhibition [6, 14].

The present analysis must obviously be considered merely preliminary: many other brain regions remain to be explored and they must all be retested with different doses and using rostro-caudal and the mediolateral gradients.

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