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Analgesia and increases in limbic and cortical MOPEG-SO_4 produced by periaqueductal gray injections of morphine

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Analgesia and changes in limbic and cortical concentrations of the major brain noradrenaline metabolite, 3-methoxy-4-hydroxy-phenylethylene glycol sulphate (MOPEG-SO_4), were investigated in rats following the bilateral injection of morphine into the periaqueductal gray (PAG). Morphine, at a dose of 5 μg per bilateral site, produced a significant antinociceptive effect within 15 min of injection. This effect, as measured by the tail flick analgesic test, remained constant at a level of approximately 75% of the maximum for 60 min. Significant increases in limbic and cortical MOPEG-SO_4 were also observed 15, 30 and 60 min after the 5 μg bilateral PAG injection of morphine. However, MOPEG-SO_4 concentrations exhibited a sharp peak in both brain areas at 30 min. Analgesia and the regional increases in MOPEG-SO_4 were antagonized by the prior systemic injection of naloxone (1 mg kg^{-1}, i.p.). Thus, analgesia and increases in noradrenaline metabolism in two brain regions appear to be mediated by the specific activation of opiate receptors in the PAG. Although these findings indicate that brain noradrenergic systems may be involved in the mediation of morphine analgesia, the lack of a strict temporal relationship between antinociceptive action and increases in MOPEG-SO_4, suggests that analgesia cannot be totally attributed to changes in brain noradrenergic transmission.

Several recent studies have demonstrated the importance of the mid-brain periaqueductal gray (PAG) in the analgesic action of morphine (Jacquet & Lajtha 1976; Yaksh et al 1976; Lewis & Gebhart 1977). The direct microinjection of morphine (1–20 μg) into this brain site elicits a dose-dependent analgesic response which is rapid in onset and detectable by several analgesiometric tests. Further, naloxone antagonism of the effects of morphine microinjection and the high density of opiate receptor sites in this brain region (Kuhar et al 1973) indicate that morphine analgesia is elicited by the specific activation of opiate receptors in the PAG. However, neurochemical changes in monoaminergic transmitter systems which may be involved in the mediation of opiate analgesia have not been previously reported following the PAG injection of morphine.

Recent investigations in our laboratory (LoPachin & Reigle 1978; Reigle & Huff 1980; Huff & Reigle 1980) have evaluated changes in brain noradrenaline turnover and metabolism produced by systemic injections of opiates and have demonstrated the ability of these drugs to produce dose-dependent increases in MOPEG-SO_4, the major metabolite of noradrenaline in brain (Schanberg et al 1968). Opiates exhibit stereospecificity, naloxone antagonism, single-dose tolerance and characteristic potency differences in producing this effect and the increases in MOPEG-SO_4 do not occur as a result of interference with the transport of this metabolite from brain. Thus, considerable evidence has been accumulated to indicate that an increase in brain noradrenaline metabolism or turnover is a specific receptor mediated effect of opiate analgesics.

Although the time course of increases in brain noradrenaline metabolism produced by systemic opiates has been shown to parallel the time course of analgesia (LoPachin & Reigle 1978), the relationship between these two opiate effects is not entirely clear. If brain noradrenergic systems are involved in the mediation of opiate analgesia, further evidence for this involvement would be obtained from a demonstration of changes in noradrenaline metabolism in selected brain regions following the injection of opiates into the PAG. As an initial attempt to provide such evidence, the following study was designed to evaluate the time course of analgesia and changes in cortical and limbic MOPEG-SO_4 produced by the injection of morphine into the ventral PAG. The morphine dose and injection site were based on those previously reported to elicit a selective analgesic response (Sharpe et al 1974).

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MATERIALS AND METHODS

Male Sprague-Dawley rats (Holtzman) with an initial weight of 200–300 g were anaesthetized with sodium pentobarbitone (40 mg kg^{-1} i.p.). Animals were placed in a stereotaxic instrument (Kopf, 900) and stainless steel guide cannulae (26 gauge) were bilaterally implanted. Guide cannulae were permanently affixed to the skull through the use of surgical screws and dental cement and were occluded with 33 gauge stainless steel wire stylets. Following surgery, animals were permitted a recovery period of at least one week, during which they were individually housed at 22 ± 2 °C with free access to food and water.

Analgesia was measured by a modification (Bass & Vander Brook 1952) of the tail flick procedure D'Amour & Smith (1941). The intensity of the focused heat stimulus was adjusted to produce a baseline (BL) latency of 3–4 s and a 12 s cut off was employed in the absence of a tail flick response. The degree of analgesia (DA) following morphine was expressed as a percentage derived from the ratio of observed change in response time (T) from baseline to the maximum possible change according to the following formula (Mayer & Hayes 1975): DA = 100(T-BL)/(12-BL). The mean and standard error of these ratios were calculated through the use of an arcsine transformation (Sokal & Rohlf 1969). Significant differences between drug and control injections or between groups killed at various times after morphine were determined by analysis of variance and Student's *t*-test ($\alpha = 0{\cdot}05$) on the transformed data.

Injections were made through 33 gauge stainless steel cannulae which were inserted into the permanent guides and extended 1 mm below their tips into the intended PAG injection sites (AP − 0·4, L ± 0·7, H − 2·2; Pellegrino et al 1979). Injection cannulae were connected to 10 µl Hamilton syringes which were held in an infusion pump (Sage 351) calibrated to deliver 0·5 µl of solution min^{-1}. The injection volume was limited to 1·0 µl per bilateral site in order to minimize diffusion and tissue damage (Jacquet & Lajtha 1976). Morphine sulphate was dissolved in sterile water for injection and drug solutions were made isotonic by the addition of sodium chloride. Morphine and 0·9% NaCl (saline) control solutions were filtered immediately before injection to eliminate bacterial contamination.

Animals were injected with a 5 µg dose of morphine (free base) per bilateral PAG site immediately following the determination of baseline tail flick latency. The analgesic response was measured 15, 30 and 60 min after injection and animals were decapitated immediately following analgesia testing. Following death, 1·0 µl of Evans blue dye was injected through the cannula implants to aid in identification of the injection site and provide an indication of the degree of diffusion. Brains were then removed and dissected according to the procedures of Glowinski & Iversen (1966) and Bartholini et al (1975). Midbrain-pontine tissue blocks were preserved in 10% formalin and subsequently embedded in celloidin, sectioned at 30 µm, stained with haematoxylin-eosin and microscopically examined for verification of the injection site (Lauber 1970).

The limbic system (mean wt = 363 ± 5 mg), which consisted of all tissue anterior to a vertical section at the rostral tip of the optic chiasm minus the corpus striatum, and the remainder of the cortex (642 ± 7 mg), were assayed for MOPEG-SO_4 according to a modification (Roffman et al 1975) of the fluorometric procedure of Meek & Neff (1972). Dissected tissue from a single animal provided sufficient material for the assay. MOPEG-SO_4 concentration was expressed as pmol g^{-1} tissue (wet weight) and means calculated from individual limbic and cortex samples were compared across times or to saline controls by analysis of variance and Student's *t*-test ($\alpha = 0{\cdot}05$).

RESULTS

Histological examination of midbrain-pontine tissue sections revealed that nearly all injection sites were located in the ventrolateral PAG and bounded by the following coordinates from the atlas of Pellegrino et al (1979): AP − 0·8 to + 0·4; L ± 0·1 to ± 1·0; H − 1·4 to − 2·6. Those few animals having injection sites not located within this area were not included in the analysis of analgesia or changes in MOPEG-SO_4.

The bilateral injection of 5 µg morphine into the PAG elicited a marked analgesic response which was apparent within 15 minutes of injection and lasted for at least 60 min (Fig. 1). Analysis of these data revealed that the degree of analgesia at all examined times following morphine was significantly ($P < 0{\cdot}01$) greater than that measured 30 min after the PAG injection of an identical volume (1·0 µl) of saline. The analgesic effects of morphine averaged about 75% of maximal at all examined times and were not significantly different from each other.

Significant ($P < 0{\cdot}05$) increases in limbic and cortical MOPEG-SO_4 were also observed in these same animals at all examined times following the 5 µg bilateral PAG injection of morphine (Fig. 2).

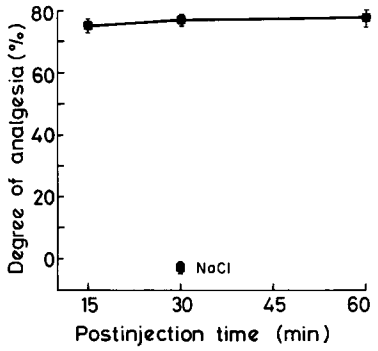

FIG. 1. Analgesic effects of 5 μg bilateral PAG injections of morphine at various times after administration. Each point represents the mean ± s.e.m. of 6–10 experimental subjects. Analgesia at all times following morphine was significantly ($P < 0.01$; transformed data, Sokal & Rohlf 1969) greater than that observed 30 min after the PAG injection of an identical volume (1·0 μl) of saline (NaCl).

However, the characteristics of these time-response curves were clearly different from those obtained for the analgesic effect. A significant ($P < 0.05$) peak in MOPEG-SO_4 concentration was observed in both brain areas 30 min after morphine indicating that the rate of onset of the maximum neurochemical change was less rapid and the duration less prolonged than the maximal analgesic response. MOPEG-SO_4 concentrations in the limbic system and cortex of animals subjected to analgesic testing and death 30 min after the PAG injection of saline were not significantly different from those observed in intact, untested controls (377 ± 17 vs 392 ± 7 and 346 ± 13 vs 364 ± 9 pmol g^{-1}, respectively).

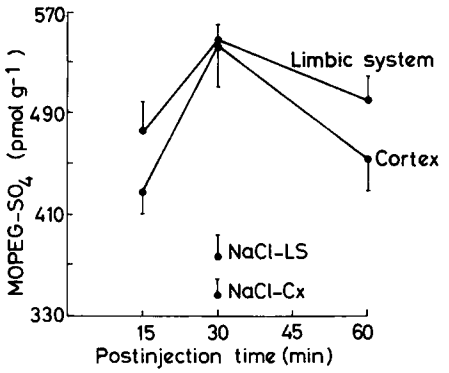

FIG. 2. Time-response curves for the effects of 5 μg bilateral PAG injections of morphine on MOPEG-SO_4 concentrations in the limbic system (LS) and cortex (Cx). Each point represents the mean ± s.e.m. of 6–9 determinations. The effects observed in both brain areas at all times following morphine were significantly ($P < 0.05$; $P < 0.01$) different from those obtained 30 min after the PAG injection of an identical volume (1·0 μl) of saline (NaCl).

To provide evidence for opiate receptor mediation of the analgesia and increases in MOPEG-SO_4 produced by PAG injections of morphine, a group of animals was pretreated with naloxone (1 mg kg^{-1}, i.p.) 10 min before bilateral PAG morphine (5 μg) injection and killed following analgesic testing 30 min after opiate administration. This dose of naloxone was sufficient to abolish the analgesia and increases in limbic and cortical MOPEG-SO_4 produced by 5 μg PAG morphine (Table 1). Tail flick latencies and MOPEG-SO_4 concentrations in these naloxone-treated animals were not significantly different from those observed in animals receiving PAG injections of saline.

Table 1. Naloxone antagonism of analgesia and increases in limbic and cortical MOPEG-SO_4 produced 30 min after 5 μg bilateral PAG injections of morphine.

Response	PAG saline	PAG morphine	Naloxone + PAG morphine*
Degree of analgesia (%)	−3·1 ± 0·3 (7)†	77.2 ± 0·6 (10)	1·8 ± 0·5 (8)
Limbic MOPEG-SO_4 (pmol g^{-1})	376·9 ± 16·6 (7)	548·3 ± 12·1 (8)	384·7 ± 10·9 (8)
Cortical MOPEG-SO_4 (pmol g^{-1})	346·4 ± 12·8 (7)	543.4 ± 32·7 (6)	355.5 ± 9·1 (8)

* Naloxone (1 mg kg^{-1}) was administered intraperitoneally 10 min before the PAG injection of morphine.

† Means ± s.e.m. followed by number of animals in parentheses. Values obtained with animals receiving naloxone plus morphine were not significantly different from those receiving saline, but were significantly different from those receiving morphine for all measured responses ($P < 0.01$).

Almost all animals were behaviourally sedated following bilateral injections of this dose (5 μg) of morphine into the PAG. However, hyperactivity was occasionally observed and was associated with cannula placements in the medial-lateral PAG. A few animals also exhibited vigorous circling behaviour when malfunction of the infusion system or aberrant cannula placement resulted in unilateral PAG injections. The circling was contralateral to the injection site. Animals exhibiting hyperactivity or circling were not included in the analysis of analgesia or changes in MOPEG-SO_4 described above.

DISCUSSION

In agreement with previous reports (Sharpe et al 1974; Yaksh et al 1976), 5 μg morphine injected into the ventrolateral PAG has been found to produce a rapid and prolonged analgesia in rats. In addition, significant increases in limbic and cortical MOPEG-SO_4 have been found to occur during the course of the analgesic response. Since brain MOEPG-SO_4 concentrations have been shown to reflect noradrenergic neuronal activity (Korf et al 1973), these findings strongly suggest that limbic and cortical noradrenergic systems participate in the mediation

of morphine analgesia. The ability of naloxone to antagonize the analgesia and increases in MOPEG-SO_4 produced by PAG morphine further supports this conclusion by indicating that both effects are elicited by the specific activation of opiate receptors.

Lomax (1966) has shown that more than 90% of a 1 µl intracerebral injection of radioactive morphine is retained within one millimeter of the injection site and a comparable degree of diffusion was observed in the present study following a 1 µl injection of Evans blue dye. Thus, it is unlikely that the effects observed are due to an interaction of morphine with brain structures located outside the PAG. Such reasoning strongly indicates that opiate sensitive neuronal elements within the PAG are capable of modulating the activity of brain noradrenergic systems. An anatomical basis for this interaction has been provided by the location of cell bodies and fibres of the periventricular noradrenergic system within the PAG (Lindvall & Bjorklund 1974) and several demonstrations of enkephalin immunoreactive neuronal elements in this same midbrain structure (Simantov et al 1977; Watson et al 1977). Although these findings may suggest a direct interaction of opiates with noradrenergic systems in the production of analgesia, the possibility of an indirect action mediated by the involvement of other transmitters can also be envisaged. For example, 5-hydroxytryptaminergic neurons originating from raphe nuclei within the PAG may also be involved in opiate action (Yaksh et al 1977) and may recruit the participation of noradrenergic systems. Additional studies are needed to clarify these potential interactions within the PAG. However, recent studies in our laboratory have shown that analgesia and increases in brain MOPEG-SO_4 produced by systemic morphine can be antagonized by the inhibition of brain 5-HT synthesis (Reigle & Barker, unpublished).

Although brain MOPEG-SO_4 levels have been directly related to noradrenergic neuronal activity (Korf et al 1973) and evidence has been advanced that MOPEG-SO_4 is formed extraneuronally (Braestrup & Nielsen 1975), increases in the concentration of this metabolite are not directly indicative of the degree of interaction of noradrenaline with its post-synaptic receptors. Increases in brain MOPEG-SO_4 could result from an increased intraneuronal deamination of noradrenaline with the major intraneuronal metabolite, 3,4-dihydroxyphenylethylene glycol (DOPEG), as an intermediate or through an increase in the formation of normetanephrine by postsynaptic *O*-methylation (Axelrod 1966). Thus, elevated MOPEG-SO_4 levels could reflect either a decrease or an increase in the interaction of noradrenaline with its postsynaptic receptors. This creates an uncertainty concerning the possibility of an activation or depression of noradrenergic activity by morphine within the PAG. Recent studies have shown that systemic morphine does not elicit an increase in brain DOPEG-SO_4 (Huff & Reigle 1980), which suggests that the opiate increases extraneuronal noradrenaline metabolism subsequent to transmitter release. This is supported by findings of increased normetanephrine in the spinal cord after morphine (Takagi et al 1979) which is also indicative of an enhanced noradrenaline-receptor interaction (Axelrod 1966). However, in vitro studies with brain tissue slices or synaptosomes have indicated that opiates produce either no effect or a decrease in spontaneous, electrically evoked or potassium stimulated noradrenaline release (Clouet & Williams 1974; Montel et al 1974; Arbilla & Langer 1978).

The results of the present study provide substantial evidence for the involvement of brain noradrenergic systems in the mediation of opiate analgesia but the nature of this involvement is not entirely clear. The lack of a strict temporal relationship between the antinociceptive effect of PAG morphine injections and increases in limbic and cortical MOPEG-SO_4 suggests that analgesia can not be totally attributed to changes in brain noradrenergic transmission and ample evidence clearly exists for the involvement of other transmitter systems (Mayer & Price 1976). However, effects on noradrenergic neurons resulting in the initiation of analgesia could occur before the manifestation of these effects as maximal increases in limbic and cortical levels of the major metabolite and, once initiated, analgesia could be maintained with a reduced or absent noradrenergic influence. Significant increases in limbic and cortical MOPEG-SO_4 were observed at all times examined following PAG morphine, although maximal changes did not follow the time course of the analgesic response. A further characterization of the increase in brain MOPEG-SO_4 and its relationship to opiate effects on other transmitter systems following PAG administration will be required to develop a more complete understanding of the neuronal events involved in the mediation of opiate analgesia.

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