Paradoxical Effects after Microinjection of Morphine in the Periaqueductal Gray Matter in the Rat

Abstract. *Paradoxical, concurrent hyper- and hyporeactivity of a profound nature to specific stimuli occurred when 10 micrograms of morphine was microinjected bilaterally into the periaqueductal gray matter of the rat brain. Both effects at this site were dose-dependent. The hyperreactivity (to previously neutral auditory and visual stimuli) was obtained only with intracerebrally injected morphine and never with intraperitoneally injected morphine or with other opiates administered either way. Rapid tolerance to toxic doses of morphine developed at this site, as well as cross tolerance of the hyporeactivity to painful stimuli between routes (intracerebral to intraperitoneal) of morphine administration. Both the hyper- and hyporeactivity were fully reversible by intracerebral injection of naloxone in the periaqueductal gray. Thus, the periaqueductal gray appears to be a major pathway for morphine action.*

Morphine microinjection in certain subcortical sites in the rat brain results in either hyper- or hypoalgesia, while other sites yield no effects (*1*). We have now obtained striking effects after microinjection of morphine in the periaqueductal gray matter (PAG) of the rat, leading us to view the PAG as a major site of morphine action.

Initially, we reported that 10 μg of morphine in this site produced extreme hyperreactivity to foot shock in the flinch-jump test; after intracerebral (IC) administration of morphine, rats reacted explosively to the foot shock; the rats made rapid and repeated high leaps (60 cm) and ran wildly about, often with severe injuries resulting. These rats appeared extremely "fearful," uttered shrill distress cries, and showed autonomic activation with shallow, rapid respiration and increased heart rate. These striking effects, however, masked other concurrent effects of morphine at this site; that is, profound hyporeactivity to such normally painful stimuli as hemostat pinch of the limbs and tail, pinpricks, ear pinches, and cold stimuli; nevertheless, other types of noxious stimuli (such as a hot plate stimulus) evoked normal reactivity.

We have developed a test battery to measure these paradoxical effects of morphine at this site. For these PAG animals, we abandoned the flinch-jump test since these animals would typically jump wildly about in the test chamber, injuring themselves severely. The test for hyperreactivity, performed at 5, 10, and 15 minutes after IC injection, consisted of a loud noise (handclap), a sudden movement within the visual field of the animal, and light tactile stimuli (air puffs); the animal was placed in a tall plastic bin for this test to avoid self-inflicted injuries that might occur during this test. The tests for hyporeactivity to painful stimuli were given 15, 60, and 180 minutes after IC administration. Table 1 shows the scoring system used for the tests of hyper- and hyporeactivity. For the latter tests, the animal was held firmly but gently. For both the hot and cold plate tests, the animal was held gently suspended over the plate by the experimenter, and either the forepaws or the hindpaws, separately, were placed on the plate, and the withdrawal latencies were noted (*2*). The details of the IC microinjection method have been described (*3*).

In the first study we localized the site of this paradoxical action of morphine where both hyper- and hyporeactivity to specific stimuli were obtained (Fig. 1). This site of paradoxical action appears to be limited only to medial sites surrounding the rostral and midportion of the aqueduct, the outermost areas of the PAG being excluded (*4*). The hyporeactive component of this syndrome was less confined in that the hyperreactivity could be obtained throughout the PAG from more caudal as well as more ventral or dorsal sites.

Next, a dose-dependent relation was found between the IC morphine dose and the concurrent hyper- and hyporeactivity (Fig. 2). At doses of 5.0 and 10 μg (given in the two bilateral sites combined), rats showed only moderate hyper- and hyporeactivity. At 20-μg doses, rats showed the full-blown syndrome, in that they made repeated and frantic high leaps (60 cm) and uttered shrill distress vocalizations to auditory or visual (or both) stimuli, although they remained profoundly hyporeactive to pinch, pinpricks, and the cold plate. (There was little overt difference between unilateral and bilateral administration of the dose as long as the total dose remained the same.) The hyperreactivity to auditory or visual stimuli was obtained only with IC morphine, and never with other opiates (etorphine, levorphanol, and methadone) administered IC or intraperitoneally (IP); nor was hyperreactivity obtained with IP morphine. The hyporeactivity to painful stimuli occurred after either IC or IP administrations of morphine, but was considerably attenuated after etorphine, levorphanol, and methadone given IC, in doses estimated from systemic studies to produce strong analgesia.

Tolerance to a toxic dose of morphine at this site developed rapidly. A single IC administration of morphine conferred tolerance to a subsequent toxic or near-toxic IC dose of mor-

Table 1. Scoring system for test battery measuring hyper- and hyporeactivity.

Stimulus	Response	Score
	Hyperreactivity	
Auditory, visual, or light tactile	Startle (with rigidly extended hindlegs)	1 point
	Backward rotation	2 points
	Leap	3 points
	Hyporeactivity	
Hemostat pinch to ears, four limbs, and tail	No response	1 point each; maximum 7 points
Pin pricks to ventral quadrants of body	No response	1 point each; maximum 4 points
Hot plate		
Forepaws	Withdrawal latency > 10 seconds	2 points
Hindpaws	Withdrawal latency > 10 seconds	2 points
Cold plate		
Forepaws	Withdrawal latency > 60 seconds	2 points
Hindpaws	Withdrawal latency > 60 seconds	2 points
		Maximum total = 19 points*

*Maximum total refers only to hyporeactivity score.

phine. No animal died of an overdose with 20 μg of morphine if that dose was preceded by a prior dose of 10 μg of morphine, whereas the death rate with an initial dose of 20 μg of morphine at this site was roughly 10 percent. An initial dose of 40 μg of morphine resulted in a fatality rate of more than 50 percent, whereas if preceded by an initial dose of either 5, 10, or 20 μg of morphine, the probability of death resulting from a dose of 40 μg of morphine was reduced to zero.

Similarly, IC morphine produced tolerance to the hyporeactivity to painful stimuli resulting from IP morphine. Only one or two prior IC doses of 20 μg of morphine resulted in animals becoming tolerant to the analgesic effects of IP morphine at 20 mg/kg.

However, only weak cross tolerance to IP doses of levorphanol (10 mg/kg) and methadone (20 mg/kg) occurred in animals with prior IC injections of morphine in the PAG. That is, hyporeactivity to painful stimuli resulting from IP levorphanol at 10 mg/kg (levorphanol is an opiate reputed to be ten times as potent as morphine via the systemic route) or IP methadone at 20 mg/kg was only slightly attenuated when preceded by IC morphine administration in the PAG.

Surprisingly, IC administrations of levorphanol (40 to 80 μg), etorphine (0.2 μg), and methadone (80 μg) had only moderate analgesic effects in the PAG (*5*). This moderate action was not due to the development of tolerance, since, in some cases, these drugs were the first given. Dextrorphan, the analgesically inactive enantiomer of levorphanol, had no effects whatsoever. Yet, 20 μg of morphine in the PAG in these same animals produced the full-blown PAG morphine syndrome.

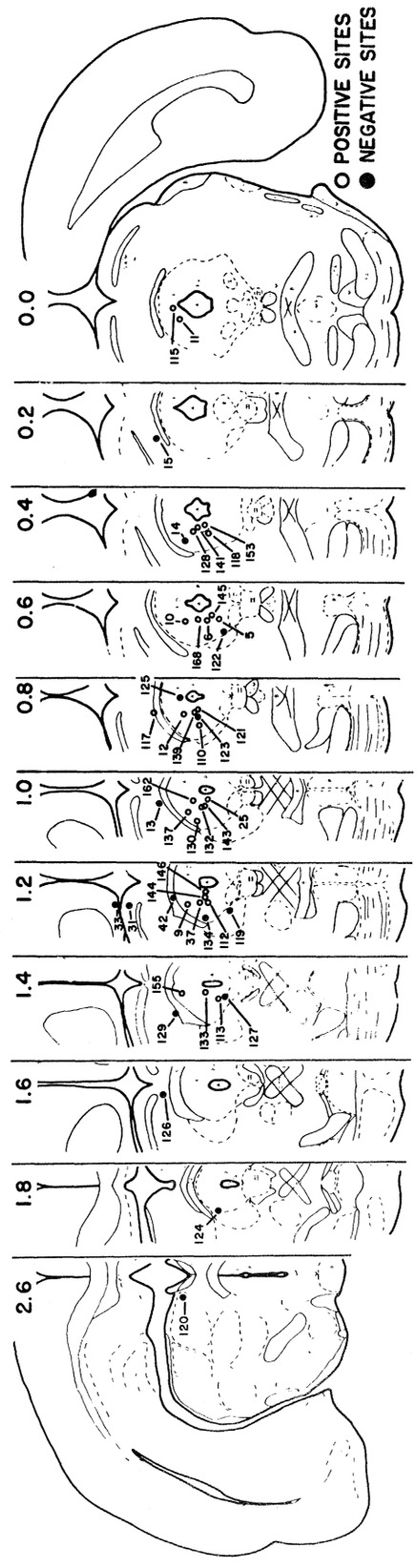

Fig. 1. Histological localization of 46 PAG sites based on blue dye (0.5 μl) injected just prior to killing the experimental animals. In some cases, injections occurred after death (if the animal died of an overdose), or no dye injection was possible if the animal tore off his cannula assembly during hyperreactivity. Spread of dye generally was limited to an area approximately 0.5 mm in width, 0.8 mm in height, and between 1.0 and 1.5 mm along the anterior-posterior dimension. Positive sites (open circles) are those sites from which hyperreactivity was obtained after a bilateral IC dose (20 μg) of morphine (which was always accompanied by strong hyporeactivity to noxious stimuli). Negative sites (closed circles) are those sites not producing hyperreactivity after a bilateral IC dose (20 μg) of morphine, but in many cases showing hyporeactivity to noxious stimuli of varying degrees. The coronal representations of the rat brain were obtained from Pellegrino and Cushman (*16*).

Reversal of both the hyper- and hyporeactivity resulting from morphine in the PAG could be obtained temporarily by an IC injection of 40 μg of naloxone. However, this reversal was short lived (about 10 minutes). Similarly, if an injection of 20 μg of naloxone preceded 20 μg of morphine, the PAG syndrome (both the hyper- and hyporeactivity) was blocked, but only for about 10 minutes. Also, the hyporeactivity to painful stimuli and catatonia resulting from 10 mg of IP levorphanol per kilogram of body weight could be reversed instantaneously for approximately 10 to 15 minutes by injection of 40 μg of naloxone administered bilaterally at the PAG site. The hyporeactivity to painful stimuli and catatonia resulting from etorphine administered IC at a dose of 2 μg or IP at a dose of 1 mg/kg could also be temporarily reversed by IC naloxone (40 μg), and more permanently by IP naloxone (10 mg/kg).

The time course differed for the hyper- and hyporeactivity resulting from IC morphine at the PAG. The onset of the hyperreactivity to auditory or visual stimuli usually occurred within 5 minutes and reached a peak at about 10 to 15 minutes. This hyperreactivity was invariably followed by a period of quiescence and catalepsy which lasted for about 1 hour. The hyporeactivity to painful stimuli took longer in onset, usually about 10 minutes, and reached a peak within 1 hour (see Fig. 2), but there was still appreciable residual hyporeactivity to painful stimuli, even after 3 hours.

The neuroanatomy of the PAG has yet to be fully elucidated. Histological studies (*6*) have found that many of the fibers coursing through the PAG terminate in the posterior hypothalamus (PH). The significant analgesia obtained in our previous study (*1*) after IC morphine in the PH, and obtained in the present studies in the PAG, appear to be morphine action on the same pathway. The concurrent hyperreactivity to auditory or visual stimuli, however, may arise from an overlapping pathway related to those subcortical sites (caudate, septum) where hyperreactivity after morphine microinjection has been observed (*1*). There are numerous reciprocal interconnections between the midbrain central gray matter and the limbic structures of the forebrain, with the hypothalamus viewed as an intervening connection between the two.

Electrical stimulation of this area has been reported (*7, 8*) to produce profound analgesia, to the extent of permitting surgery without anesthesia in the rat. We also were able to excise a small tumor from the dorsal region of the neck in a rat treated with 20 μg of bilateral morphine in the PAG without any sign of pain. However, there has been no previous mention of any concurrent hyperreactivity of the kind observed with IC morphine when the same area was electrically stimulated (*9*).

Recent reports have suggested that the opiate receptor has been identified in the rat (*10*) and primate (*11*), and partially purified in the mouse (*12*). It was found that such receptors occurred in high concentration in the striatum of the rat, and in the anterior amygdala of the primate and human brain. The PAG had moderate concentrations of the receptor (half that of the anterior amygdala) (*11*). In these investigations it was assumed that affinity for opiate receptor binding paralleled analgesic potency of opiates after systemic administration, and that such affinity would be stereospecific. Thus, levorphanol bound more of these receptors than its analgesically inactive enantiomer, dextrorphan, as was indicated by subsequent binding of [^{3}H]naloxone in brain homogenates. Hiller *et al.* (*13*), using a similar technique with etorphine, obtained similar findings, with the highest concentrations of the receptor occurring in the amygdala and the septum, and moderate concentrations in the PAG and the hypothalamus. These sites of high opiate receptor concentration do not show strong correlation with the "analgesic" sites identified in our studies with IC morphine. This discrepancy may be due to the possibility that such opiate receptors mediate other general effects of morphine.

In addition, our results indicate that relative analgesic potencies of opiates after IC administration do not parallel their analgesic potencies after systemic administration. This is similar to results obtained by Kutter *et al.* (*14*), who compared the minimum effective dose necessary to achieve analgesic action when opiates were administered by the intravenous or intraventricular routes. By the intravenous route, etorphine, levorphanol, and methadone required 1/5000, 1/11, and 1/5, respectively, of the morphine dose, to achieve

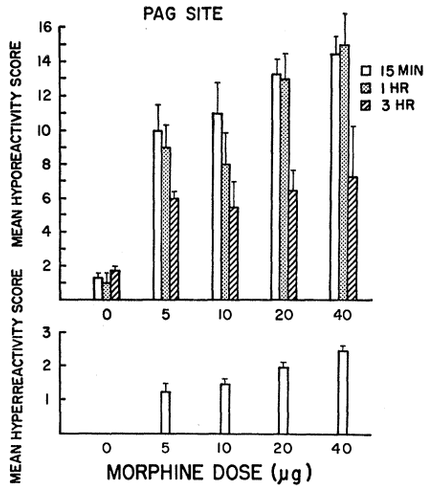

Fig. 2. Dose-related scores of hyper- and hyporeactivity of PAG animals after IC doses of morphine. The hyperreactivity was measured at 5, 10, and 15 minutes after IC administration, and the highest score achieved (1, 2, or 3) during this interval was assigned to the animal. The hyporeactivity was measured at 15, 60, and 180 minutes after IC administration. Separate groups are shown at each morphine dose, each group consisting of four to six animals. (The injection volume was always 0.5 μl for each bilateral site.)

comparable analgesic results; by the intraventricular route, the ratio was reduced or reversed to 1/34, 7, and 30 times the morphine dose, respectively. Apparently, in the systemic route, lipophilic compounds, such as etorphine and levorphanol, penetrate the blood-brain barrier and reach receptor sites more rapidly before being metabolized than more hydrophilic compounds such as morphine. In contrast, after IC administration, the lipophilic drugs may become inactivated and removed from receptor sites more rapidly than morphine. Kutter *et al.* also point out that polar compounds, such as morphine, have greater activity at the receptor than does methadone. Thus, analgesic potency of an opiate depends on route of administration—whether systemic, intraventricular, or intracerebral, and is a complex function of penetration through the blood-brain barrier, peripheral metabolism and elimination, and specific drug-receptor interaction.

The rapid tolerance following only one or two administrations of IC morphine indicate that tolerance is a central phenomenon. On the basis of our results, several possible factors in tolerance development can be eliminated; it is safe to say that peripheral metabolism of opiates is not involved in opiate tolerance development, nor are changes in the blood-brain barrier a possible factor here. Our results imply that changes occur locally, perhaps in the receptors themselves.

Evidence from other laboratories suggests the importance of the PAG in morphine action (*15*). The effects reported here which we have obtained after microinjection of morphine in discrete, circumscribed areas of the PAG confirm that this site is indeed an important pathway for morphine effects.

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References and Notes

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2. In general, the hot plate test (forepaws and hindpaws separately on a 55°C plate more than 10 seconds) showed positive results only when analgesia was most profound. The pinpricks and ear pinches often showed "false positive" results. The cold plate and the hemostat pinches of the four limbs appeared to be the most discriminating, with the hindpaws often being more sensitive to pinch than forepaws, at moderate levels of analgesia.
3. Y. F. Jacquet, in *Methods of Narcotics Research*, S. Ehrenpreis and A. Neidle, Eds. (Dekker, New York, in press).
4. A previous study which included numerous erroneous placements in more caudal portions of the PAG yielded hypo- but not hyperreactivity to specific stimuli.
5. When etorphine was increased to 2 μg, the animals showed strong analgesia. It was not possible to increase doses for levorphanol and methadone, however, without increasing injection volume, so higher doses for these two drugs were not used.
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9. However, Mayer *et al.* (*8*) found that some rats with stimulating electrodes in the PAG were hyperreactive to light touch, and "squeaked and struggled when picked up or gently handled during brain stimulation. Several made organized attacks on objects placed in their visual fields." In comparison, our PAG animals, although appearing extremely "fearful," never attacked the experimenter and were remarkable for their docility and nonaggressiveness.
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