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BRIEF COMMUNICATION

Localization of the Antinociceptive Action of Morphine in Primate Brain[1,2]

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PERT, A. AND T. YAKSH. *Localization of the antinociceptive action of morphine in primate brain.* PHARMAC. BIOCHEM. BEHAV. 3(1) 133–138, 1975. – Microinjections of morphine sulfate (20–40 μg) were made into various subcortical regions of the rhesus monkey brain. The effects of these injections were evaluated on the nociceptive threshold as defined by the shock titration technique. The results of this preliminary investigation indicate that the region of maximal antinociceptive sensitivity to morphine in the primate is the periventricular-periaqueductal gray matter. It is tentatively suggested that morphine lowers that affective tone or the aversive component of pain by its action on the midbrain central gray and periventricular areas – both important projection and integration areas of the extralemniscal somatosensory system.

Morphine Periventricular-periaqueductal gray matter Anatomical localization of morphine analgesia
Rhesus monkey

WORK by a number of investigators employing microinjection techniques has suggested a certain degree of localization of the site of action in brain of morphine with respect to the development of analgesia. Early studies by Tsou and Jang [35] in rabbits and later by Jacquet and Lajtha [16] in rats have strongly implicated the regions surrounding the third ventricle in the vicinity of the rostral and caudal hypothalamus. Recent work by Buxbaum *et al.* [5], however, has localized such sensitivity in the rat to the anterior nuclear group of the thalamus. In contrast to these results, Herz and his colleagues [12,13], employing tissue injections and injections into separate parts of the ventricular system, indicated that the relevant structures in the rabbit were located in the floor of the fourth ventricle. Such data have not been previously reported for higher species.

Localization of the action of morphine in the primate brain takes on special importance in light of the known differences in the neuroanatomical organization of the pain pathways among vertebrate species (e.g., specifically the neospinothalamic tract [22]) and considering the apparent difficulties of localizing the action of morphine in the small rodent brain with microinjection techniques. We present here preliminary data concerning the analgesic sites of action of morphine in the primate (Macaca mulatta) brain.

METHOD

Seven Rhesus monkeys, weighing from 4.5–5.5 kg, were acclimated to primate restraining chairs and trained on the shock titration procedure described below. After titration training, these animals, under Nembutal anesthesia (40 mg/kg), were implanted stereotaxically following aseptic procedures with arrays of 4 to 6 pairs of bilateral 22 ga stainless steel microinjection guides. After recovery the sites were mapped for antinociceptive activity by bilateral injections of 1.0 or 2.0 μl of a solution containing 20 μg of morphine sulfate in normal saline (pH = 4.9). If a bilateral injection produced an antinociceptive response, unilateral injections were made to provide a more definitive localization of the active site. To allow the maximum number of sites to be mapped, injections were made through 27 ga injection cannulae at successively greater depths past the tip of the guide cannulae in increments of 2 or 3 mm. Depending on the placement of the guide cannulae, this methodology ideally enabled us to map approxi-

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[2] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences–National Research Council.

mately 50 sites in a single animal. At least 7 days were allowed to elapse between injections through the same guide cannula. This long interval provided assurance that there was negligible interaction between injections.

The pharmacological specificity of the antinociceptive action of intracerebrally administered morphine was ascertained by two techniques. First, naloxone HCl (1.0–5.0 mg/kg), a specific opiate antagonist, was administered intravenously at the end of a session in which an antinociceptive response had appeared. Second, dextrorphan tartrate (20 μg, pH = 3.4 in normal saline), a pharmacologically inactive opiate analog, was injected into the morphine sensitive site during a subsequent session. A morphine response was considered to be pharmacologically specific only if naloxone produced a reversal of the antinociception and if an injection of dextrorphan had no effect at the morphine sensitive site.

After the series of experiments was completed, the animal was sacrificed and the sites of injection were localized according to standard histological practice.

To measure the nociceptive threshold, the shock titration technique, previously described by Weiss and Laties [36] was employed. In this paradigm electric shock, applied to the food pads of the monkey, was increased by 0.16 mA every 2 sec by a BRS titrating constant current AC shocker (No. 113-08). The animal, by responding on an available lever, could decrease the level of shock by an equal amount (0.16 mA) with each response. The maximum level of shock was set at 4.0 mA. Sodeco counters cumulated the amount of time that the animal spent at each shock level in tenths of seconds. Data were recorded in 1 hr segments and the threshold was defined as that shock level below which the animal spent 90% of an hour run. The threshold defined in this manner appears to represent an aversive threshold and not a sensory threshold (e.g., threshold for the electrical shock to act as a CS in an avoidance situation) since shock at this level will support escape behavior without extinction for extended periods of time (Pert, in preparation). An animal was typically run for at least 3 hrs following an injection. If a morphine injection proved to be effective, the session was extended to determine the longevity of the antinociceptive response. An additional performance record was obtained with a strip chart recorder (Varian Model No. 6-4000) which gave a graphic representation of the amount of shock that the animal tolerated as a function of time. These data were useful for determining the onset and duration of action of the centrally administered morphine.

An injection of morphine was considered to have been effective if the shock threshold increased by 0.64 mA or more during the course of 3 hr. Control animals kept on the titration schedule for up to 9 hr rarely demonstrated a shift in threshold of more than 0.32 mA.

When an analgesic response, as defined by the shock titration paradigm, was found, we further evaluated the generality of this effect by lightly pinching the animals' limbs and torso with a pair of forceps. Such manipulations always produced motor reactions, often accompanied by barking, in a normal animal. The pinching pressure was always kept far below a level which would have produced tissue destruction.

RESULTS

The typical effects found following injections of morphine into four regions of the rhesus monkey brain appear in Fig. 1. Unilateral injections of 20 μg of morphine sulfate into the caudate nucleus (Fig. 1A) and anterior thalamus (Fig. 1B) had no effect on the shock threshold over a period of five hours. A similar injection of morphine into the periventricular gray region of the posterior hypothalamus (Fig. 1C), however, produced an antinociceptive response which began to develop during the second hour and reached asymptote during the third hour. An intravenous injection of naloxone (5 mg/kg) at the start of the fifth postinjection hour produced an immediate reversal of the antinociceptive response. A 20 μg injection of dextrorphan (Fig. 1D), the inactive enantiomer of levorphanol, into the identical site one week later had no effect over the course of 5 hr. An injection of 10 μg of morphine into the fourth ventricle (Fig. 1E) also produced a small increase in the shock threshold which became evident during the first hour and reached asymptote during the second hour. An intravenous injection of naloxone again produced an immediate return of the nociceptive threshold to the preinjection baseline.

Out of the 217 sites which have been mapped so far, only 13 (6%) were found to be responsive to the direct application of morphine. Five out of the 6 sites located in the periaqueductal gray matter proved to be active. Six active sites were found in the medial hypothalamus (1.0–2.0 mm from midline) with a distribution from the preoptic region to the mammillary bodies. Only one site in the medial hypothalamus proved to be inactive by our criterion. The remaining two active sites were located in the ventromedial thalamus, in the region of the intralaminar thalamic nuclei. This region did not appear to be as responsive as the hypothalamus and five sites slightly more dorsal had no activity at all.

In addition to the caudate nucleus and anterior thalamus, other sites which proved to be unresponsive include the ventrobasal and ventrolateral nuclear groups of the thalamus, the globus pallidus, putamen, cortex, cerebellum, septum, superior and inferior colliculi or any fiber tracts including the corpus collosum, fornix, the anterior and posterior commissures or the mammillo-thalamic tract.

The onset of a typical antinociceptive response occurred approximately 1.5 hrs following an injection of 20 μg of morphine into an active site. This latency could be decreased (only relatively little however) by increasing the dose to 40 μg. We never observed a response earlier than an hour even following bilateral injections of 40 μg of morphine. An increase in dose also appeared to increase the magnitude and duration of a response. The typical antinociceptive response lasted approximately 5–8 hours past its initiation following an injection of 20 μg of morphine into an active site.

Intravenous injections of naloxone reversed each antinociceptive reaction produced by morphine and dextrorphan proved to be inactive at each morphine responsive site.

Mechanical pressure applied to the limbs and torso during an antinociceptive response no longer produced the typical aversive reactions seen in the normal animal. In every instance the motor reactions and barking were attenuated or eliminated altogether.

DISCUSSION

These preliminary findings indicate that the antinociceptive actions of morphine appear to be confined pri-

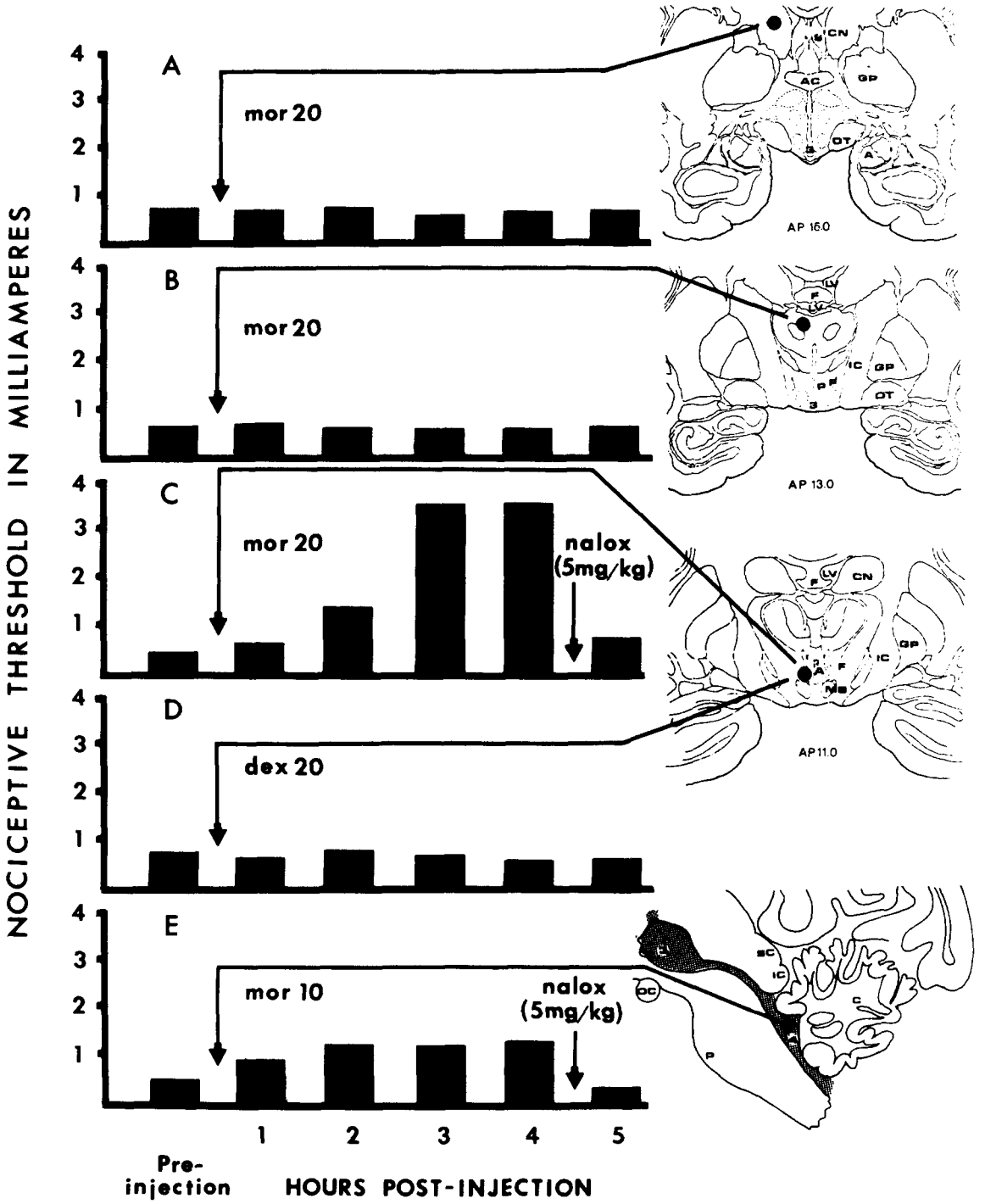

FIG. 1. Threshold expressed in mA of shock as a function of time following microinjection of morphine sulfate (20 μg) at the sites shown in the adjacent histological plates: (A) caudate nucleus, (B) anterior thalamus, and (C) periventricular gray. The bottom graph (D) represents the response in the same animal as in (C) following injection of dextrorphan (20 μg) at the identical site. Part E illustrates the antinociceptive response following an injection of 10 μg of morphine sulfate into the 4th ventricle. Anatomical abbreviations are: (A) = aqueduct; (AC) = anterior commissure; (C) = cerebellum; (CN) = caudate nucleus; (F) = fornix; (GP) = globus pallidus; (IC) = internal capsule; (MB) = mammillary body; (OC) = optic chiasm; (OT) = optic tract; (PH) = posterior hypothalamus; (S) = septum; (3) = third ventricle; (4) = fourth ventricle.

marily to the periventricular-periaqueductal regions in the primate brain. The specificity of the morphine induced antinociception was clearly confirmed by the findings that naloxone reversed each antinociceptive reaction and that dextrorphan had no effect at the identical site.

Antinociception, as measured by the shock titration technique, seems to reflect true analgesia and is apparently not determined by the effect of morphine on some sensory or motor mechanism unique to the titration paradigm. Whenever we found an increase in the shock threshold following a central injection of morphine, we also found an attenuation or elimination of the aversive reactions to mechanical pressure to various areas of the animals' body.

The slow onset of the morphine induced antinociceptive response requires comment. We feel that this effect is due to two factors. First, morphine diffuses through brain tissue rather slowly due to its relatively low lipophilic characteristic [13]. Second, a critical mass of brain tissue may have to be affected by morphine in order to produce an antinociceptive response. We estimate this area to be approximately 3 mm in diameter in the rhesus monkey since injections as close as 2 or 3 mm from the active site were often found to produce little if any effect. One to two hours are apparently required for 20 μg of morphine sulfate to diffuse in pharmacologically significnat quantities over this area.

The slow onset, of course, could also reflect the diffusion of morphine into the ventricles. However, work presently in progress with ^{3}H-morphine sulfate (Pert and Yaksh, in preparation) indicates that less than 10% of the injected dose reaches the ventricles during the first several hours after an injection 1.5 mm from the ventricle wall. Equivalent doses injected by this route have no effect on the nociceptive threshold of the animal. Furthermore, an injection of 10 μg of morphine into the fourth ventricle produced a much smaller response than that which was obtained from an injection of 20 μg into a sensitive site (Fig. 1E). These data, considered together, preclude the possibility that the intracerebrally administered morphine is acting at some other sites located along the walls of the ventricles following diffusion into the ventricular system.

Casey and Melzack [6] and Melzack and Wall [25] have proposed that the appreciation of pain by the central nervous system is governed by a complex interaction among three primary and discrete somatosensory systems which mediate different facets of pain phenomena. The significance of our findings is to be found in the relationship of the morphine sensitive sites to the neuroanatomy of these somatosensory systems in the primate.

It is significant to note that the loci which we have found to be particularly sensitive to the direct application of morphine are concentrated primarily in the periaqueductal-periventricular region of the midbrain and diencephalon. This region of the brain contains some of the terminal areas and associated structures of the extralemniscal somatosensory projection system which has been postulated to mediate the aversive or affective-motivational component of pain [1, 6, 25]. The classical lemniscal projection areas (e.g. somatosensory thalamus), which presumably mediate the discriminative components of pain, have so far proved to be relatively unresponsive to morphine. All cortical areas we have looked at, either associational or sensory, have also proved to be insensitive.

The central gray or periaqueductal gray region has been implicated in the appreciation of pain by a variety of findings. Acute electrical stimulation of this region in primates [8,9], cats [15, 32, 33] and rats [28,29] has been found to produce aversive reactions. In man, pain and aversive emotional feelings have been reported following stimulation of this region [27]. In addition, stimulation of the central gray matter has been reported to lower the electrical shock threshold of monkeys in the shock titration paradigm [30]. Flight, rage, and attack responses have been reported following the electrical stimulation of various hypothalamic areas in cats [10,32]. Significantly, a number of hypothalamic regions proved to be analgesically responsive to the direct application of morphine in our preparations.

Lesions in the central gray matter, on the other hand, have produced a significant, although sometimes transient, decrease in the capacity of cats to react to noxious stimuli [24,31]. Lesions in the central gray matter of rats have also produced deficits in escape and avoidance tasks [11,19]. Furthermore, lesions in the periaqueductal gray matter of cats have prevented the flight and rage reactions produced by stimulation of the rostral perifornical region of the hypothalamus [32] and the attack response to stimulation of the posterior midline thalamic region [2].

Evoked potentials recorded from the central gray matter and surrounding tegmental areas, following the presentation of nociceptive stimuli, have been reported to be depressed after the administration of morphine by a number of investigators [23, 26, 34]. These findings, unfortunately, have not been entirely consistent [7] and furthermore there is apparently little agreement regarding the precise relationship between this type of evoked electrical activity and pain [7,34].

Paradoxically, a number of studies have recently reported that analgesia can be obtained by prolonged electrical stimulation of the periaqueductal gray matter of rats [21] and cats [18]. It has been suggested that this stimulation induced analgesia is caused by the activation of brain stem structures which have a descending inhibitory influence on the transmission of nociceptive information through the spinal cord [18]. It is also conceivable that this high intensity prolonged stimulation produced a functional blockade of the periaqueductal gray matter or of a certain population of neurons located in this region. This hypothesis appears to be more consonant with the behavioral and physiological findings related to this area which were described above.

It is also of interest to note that the distribution of morphine sensitive sites in this study is related in some degree to the distribution of sterospecific binding of opiates in the primate brain [14,17]. Binding has been found to be the highest in limbic forebrain, limbic midbrain and medial diencephalic structures. Among the highest regions in binding of these limbic and diencephalic areas were the periventricular-periaqueductal gray matter and the hypothalamus. Significantly, however, all regions showing high opiate binding have not been observed to correlate with analgesic activity. Important examples of this are the caudate nucleus and amygdala in which bilateral injections of up to 80 μg of morphine resulted in no measureable change in threshold over 6 hours. It appears that these analgesically unresponsive areas may mediate other physiological effects of opiates or play a less important role in opiate induced analgesia.

Although the findings related to the possible role of the central gray area in the appreciation of pain suggest that inhibition of this region by such means as lesions produces

analgesia and acute stimulation produces affective aversive reactions indicative of pain, it should not be assumed that morphine acts here by a simple blockade of function in the same manner as a lesion. The periventricular-periaqueductal gray matter is a highly polysynaptic region. Any one of a number of differentially coded neurons could be the target site of opiates at this level. Morphine could facilitate a specific population of inhibitory neurons, inhibit facilitatory neurons or vice versa.

It is tentatively suggested that the regions of the hypothalamus which we found to be particularly responsive to morphine may be important sites of integration of nociceptive stimuli and translation of such stimuli into visceral responses associated with the affective component of pain. The periaqueductal gray matter may also be an important integrative center for pain or possibly an origin of descending pathways for the modulation of affective behavior associated with pain. Considering the highly polysnaptic nature of neurons in the periaqueductal gray region and its complex relationship with limbic structures, it is conceivable that this area may constitute a self-reliant system and may not be simply a center through which pathways traverse. It is suggested that morphine lowers the affective tone of pain by its action on the midbrain central gray and periventricular areas – both important projection and integration areas of the extralemniscal system.

One important difference between our findings and those of a recent study with rats should be noted. Jacquet and Lajtha [16] have reported that injections of morphine into the periaqueductal gray region of rats produces what they have termed – "hyperalgesia". This effect however, is probably not related to a decrease in the pain threshold per se but reflects nonspecific hyperreflexia produced by morphine injections into this region. Hyperreflexia and hyperactivity, concomitant with analgesia, have been reported following injections of morphine into the central gray matter [12] and ventricles [3] of rabbits and following injections into the hypothalamus [20] of rats.

We have failed to observe such hyperreflexia or hyperactivity following injections of morphine into any region of the primate brain. The reactions which have been observed in the rodent may reflect the use of a relatively high dose of morphine and its action over a larger mass of neuroanatomically analagous tissue.

With respect to other species differences (i.e. differences in the loci of action of morphine with respect to analgesia), it is not possible at this time to decide whether the observed variation is due to either true differences in the anatomy of the pain system, to the types of techniques used to study nociception (i.e. voluntary versus reflexive responses) or to the difficulties inherent in separating functions in the small rodent brain.

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