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Central and Peripheral Components of Morphine Mydriasis in Mice

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KORCZYN, A. D. AND D. MAOR. *Central and peripheral components of morphine mydriasis in mice.* PHARMAC. BIOCHEM. BEHAV. **17**(5) 897–899, 1982.—Mice treated with morphine (intracerebroventricularly, retrobulbarly or intraperitoneally) demonstrated dose related bilateral mydriasis. Intracerebroventricular (ICV) injection was much more potent than either retrobulbar (RB) or intraperitoneal (IP) injections. Morphine applied topically onto one eye resulted in bilateral mydriasis which was more marked in the treated eye. The mydriatic effect was antagonized by naloxone administered either IP or ICV or given on one eye. Here again, ICV naloxone was most effective. Naloxone eye drops diminished the mydriasis produced by systemic morphine bilaterally but more in the treated eye. These results suggest that in mice the mydriasis produced by morphine is mainly of central origin, but a local ocular effect also occurs.

Opiates Morphine Mydriasis Naloxone Pupil

THE pupillary effects of opiates have been recognized for several years. In humans, morphine causes a characteristic miosis, and this effect is also seen in rabbits and dogs. In rats and mice, however, opiates cause mydriasis [2,3]. The opiate effect is thought to be central in origin, but although support for this site of action is available for the miotic action [5], none exist as far as mydriasis is concerned. Lately, Wallenstein and Wang [7] suggested that in cats the adrenal medulla is important in morphine-mydriasis. However, their results do not bear on the question as to whether the action of opiates on the adrenal medulla is direct or mediated through the central nervous system. The present study attempted to explore, in mice, the contribution of central and local ocular effects of opiates in the production of mydriasis.

METHOD

Male ICR albino mice, Swiss strain, weighing 20–25 g were used in all experiments. Pupillary diameters were measured through a Zeiss operating microscope, with a scale in one of the oculars, as previously described [3]. For pupil measurements, mice were held under the microscope for about 10 seconds, and between measurements were in their cages.

Morphine hydrochloride (Assia) and naloxone hydorchloride (Endo) were used. The drugs were dissolved in saline and injected intraperitoneally (IP) at a volume of 5 ml/kg. Retrobular (RB) injections were done unilaterally and animals with obvious ocular damage were discarded. Intracerebroventricular (ICV) injections were performed with 10 μl Hamilton syringe 1.0–1.2 mm lateral to the crossing of the sagittal and coronal sutures (which were exposed 24 hours previously, under ether anesthesia). The needles were especially fitted with a stop which allowed them to be introduced only 2.0–2.1 mm. Following injections (10 μl volumes) the needle was left in place for about 5 sec and withdrawn. The animals were conscious during drug administration, and were allowed to recover after termination of the injections. Control groups were given saline injections at the same route and volume as the experimental groups. The accuracy of the ICV method was confirmed in a separate set of experiments in which methylene blue was injected and seen to be distributed in the cerebroventricular system.

Statistical evaluation was performed using Student's t-test and/or paired t-test, and results considered significant for p values smaller than 0.05.

In some experiments, drugs were topically applied into the conjunctival sac; 0.1 ml of either morphine (10 mg/ml), naloxone (2 mg/ml) or saline were administered unilaterally. (Since only a portion of the dose applied is absorbed through the cornea, dose-response curves were not construed).

Statistical analysis was done using two-tailed Student's t-test or paired t-test, as appropriate.

RESULTS

Injection of morphine either IP, RB or ICV produced dose-dependent mydriasis, whereas saline injections have not produced statistically significant changes of pupillary size. As shown in Fig. 1, the dose response curve for ICV injection of morphine showed a shift to the left of about two orders of magnitude. RB and IP injections were similar in their effects. Although retrobular injection of morphine was done unilaterally, the effect was always bilateral and symmetric. When morphine was applied topically onto the conjunctiva, bilateral mydriasis also occurred but it was signifi-

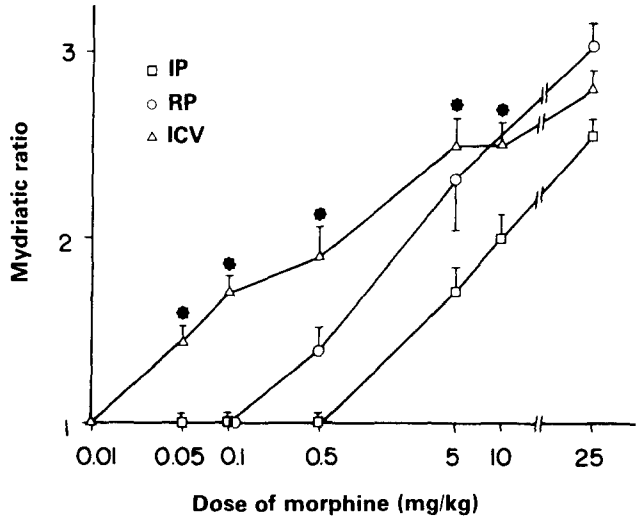

FIG. 1. Dose response curves of the mydriatic response to morphine injected intraperitoneally (IP), retrobulbarly (RB) and intracerebroventricularly (ICV). The mydriatic ratio is the ratio between the pupillary diameter following treatment and prior to drug administration. Data are means±S.E. Each point represents results from 6–48 mice. *Different from IP injected group ($p<0.05$).

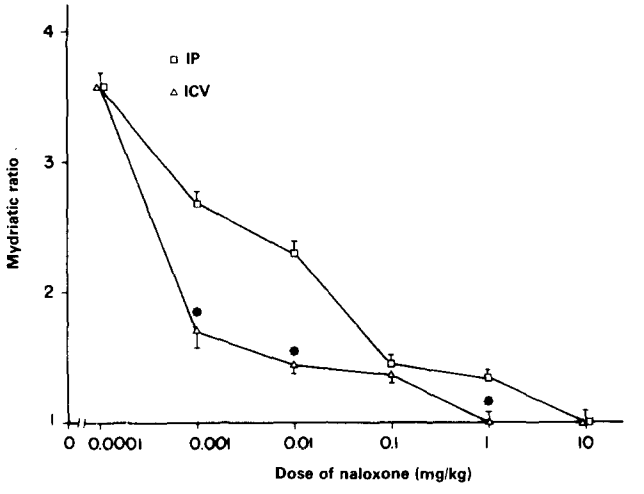

FIG. 3. The antagonism by different doses of naloxone of mydriasis produced by morphine (50 mg/kg, IP) at 60 min. Data are means±S.E. Each point represents results from a group of 6 animals. *Significantly different from animals injected IP with morphine, who were not treated with naloxone ($p<0.05$).

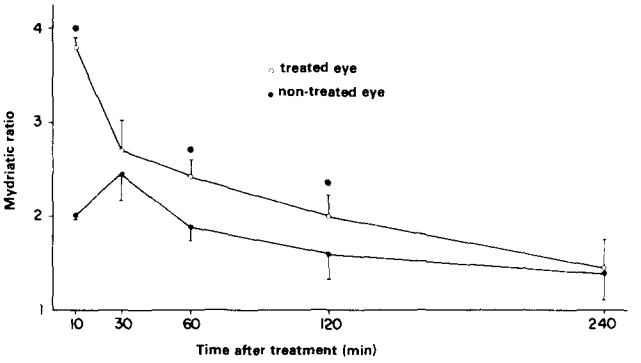

FIG. 2. The mydriatic effects of eye drops of morphine at different time intervals. Data are means±S.E. Each point represents results from a group of 5 animals. *Different from non-treated eye ($p<0.05$).

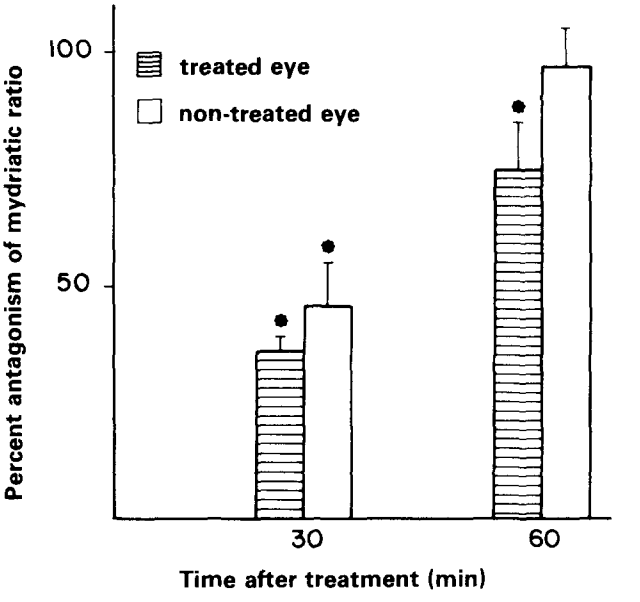

FIG. 4. Antagonism by naloxone eye drops of the mydriasis produced by systemic morphine, 30 and 60 min following morphine injection (50 mg/kg IP). Data are means±S.E. Each point represents results from a group of 5 animals. *Significantly different from animals injected IP with morphine, who were not treated with naloxone ($p<0.05$). The differences between treated and non-treated eyes were significantly different ($p<0.05$) at either 30 or 60 min.

cantly more prominent on the treated side, particularly immediately after administration (Fig. 2). Hematogenous spread to the the contralateral side as well as to the brain probably accounted for the diminution with time of the difference between the treated and non-treated eyes.

Naloxone antagonized morphine mydriasis when injected either IP or ICV. ICV injections were more effective (Fig. 3). Naloxone eye drops antagonized the mydriasis produced by systemic morphine administration. Although the antagonism was bilateral it was significantly more marked in the treated eye (Fig. 4).

DISCUSSION

The pupillary effects of opiates are easily observable and may be much less complicated actions than, for example, analgesia. However, only meager information is available concerning the mydriatic effect in rodents. The mydriasis is

clearly dose-dependent [3] and as the present results show the effect seems to be a specific opiate action in that it is antagonized by naloxone.

It is widely recognized that opiates have both central and peripheral actions, and with the recent discovery of enkephalinergic innervation these actions are becoming more closely related to the physiologic processes. However, previous work from this laboratory failed to detect a physiologic role of enkephalins in the regulation of pupillary diameter [4].

The work of Lee and Wang [5] has supported the contention that opiates affect the pupil through central actions. However, their work concentrated on dogs, in which opiates cause miosis. According to the results of the present study, the mydriasis produced by opiates in mice also has a central component. Intracerebroventricular administration was found to be much more potent than systemic injection, the latter given either IP [3] or IV [4]. Morphine is known to penetrate the blood brain barrier only slightly, and a figure of about 1% is given [1], thus agreeing with the present findings. Similarly, Naloxone given intracerebroventricularly was more efficacious in reversing morphine mydriasis (when morphine was injected systemically) and although here the ratio was smaller than 100:1, this reflects the easier penetrability of this antagonist into the brain.

We do not have data to indicate the intimate central mechanisms which are involved in opiate-induced mydriasis. We have previously studied the neuropharmacology of this action [3] and found opiate-induced mydriasis to be abolished by pretreatment with Ro 4-1284, a drug which is known to deplete nerve terminals of several transmitters.

We concluded that the opiates probably act on neurons which release an unidentified transmitter, not likely to be a catecholamine or indoleamine, since blockade in the synthesis of these neurotransmitters or the application of their respective receptor antagonists did not influence morphine mydriasis.

In addition to the central effect of opiates, morphine seems to have a local effect on the eye. Topical application of the drug onto the conjunctiva unilaterally produced an asymmetrical effect, with a more marked ipsilateral mydriasis. Also, a local application of naloxone after systemic morphine injection was more potent ipsilaterally in reversing the mydriasis caused by systemic morphine. The contralateral effects probably result from the systemic absorption of the drugs, which is known to occur after ocular administration. Retrobulbar injection failed to cause an asymmetric response, probably because of rapid systemic absorption. However, the asymmetric component following topical application is probably due to direct action on the eye.

A local ocular component of opiate-induced miosis has previously been tested [5], but the parallel possibility for animals in which opiates produce mydriasis has never been investigated, and in fact was first demonstrated by our experiments. Based on analogy with other systems [6], we propose that this action may occur through interference with the release of acetylcholine from the terminals of the oculomotor nerves either in the ciliary ganglion or in the terminals of the ciliary nerves in the iris. Our results indicate that the contribution of this component to the mydriasis is small, with the main effect exerted within the central nervous system.

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