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ANTAGONISM OF OPIATE MYDRIASIS IN MICE

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1 Morphine-induced mydriasis in mice is antagonized by nalorphine, levallorphan and naloxone in a dose-dependent manner.

2 The relative potency of the three agents is 10: 56: 134 respectively, thus being in accordance with other tests of narcotic antagonism. Naloxone has the shortest duration of action.

3 When injected into naive animals, nalorphine (but not levallorphan or naloxone) produces a slight mydriasis.

4 Measurement of the diameter of the pupil in mice seems to be a precise, simple and rapid test for studying narcotic antagonist as well as agonist action and has several advantages over standard methods used for this purpose.

Introduction

Opiates have characteristic effects on the pupil, although these differ markedly between species. In some animals, including the dog, rabbit and human, opiates produce miosis, while in most others, mydriasis occurs (Klemfuss, Tallarida, Adler & Adler, 1978). This mydriasis seems to be closely related to other specific opiate action. For example, Janssen & Jageneau (1956) have shown in mice a high positive correlation between the mydriatic action and the analgesic activity of a large series of opiate derivatives. This contrasts with the lack of correlation between analgesia and miosis in man (Fraser, Nohs, Vanhorn & Isbell, 1954).

In the present work we have analysed the effects of three narcotic antagonists, nalorphine, levallorphan and naloxone on morphine-induced mydriasis in mice. Our aim was to establish whether the pharmacokinetic effects of these agents are similar to those which have been reported for other specific opiate actions.

Methods

Naive male ICR albino mice (Swiss strain), weighing 20–40 g were used. Pupil diameters were measured with a magnification of ×20 as previously described (Korczyn, Boyman & Shifter, 1979). All drugs were injected intraperitoneally to groups of 8–10 mice. Morphine hydrochloride was given at a dose of 100 mg/kg, producing maximal mydriasis (Korczyn *et al.*, 1979), and 30 min later one of the antagonists was injected. The doses used were: nalorphine hydro-

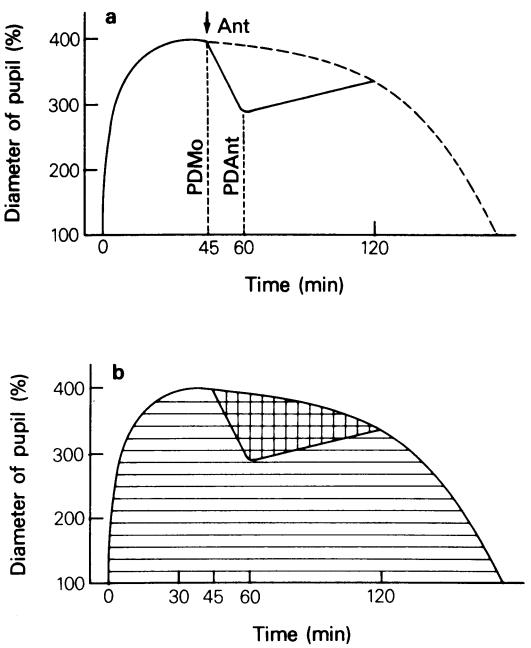

Figure 1 (a) Diameter of pupil (%) after treatment with morphine and antagonized by an opiate antagonist given at the peak effect of morphine (30 min after i.p. injection). PD_{Mo} is the diameter 30 min after morphine injection; PD_{Ant} is the diameter at the peak antagonist action in morphine-treated mice. (b) Reduction of area under curve (AUC) after treatment with morphine and antagonized by an opiate antagonist given at the peak effect of morphine. Hatched area, morphine effect. Cross hatched, antagonist effect.

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chloride 2.5, 5, 10, 25, 50, 100, 250 or 500 mg/kg; naloxone 0.25, 0.5, 1, 2, 2.5, 5 or 10 mg/kg; levallorphan 1, 2.5, 7.5, 20, 50, or 100 mg/kg. Each combination was given to a separate group of naive animals, and control groups were always injected with 0.9% w/v NaCl solution (saline) instead of the antagonists. Maximal inhibitory effects (MIE) of the antagonists against morphine-induced mydriasis were calculated for each treatment according to the following formula (Figure 1a):

$$\frac{PD_{Mo} - PD_{Ant}}{PD_{Mo}} \times 100$$

PD_{Mo} is the diameter 30 min after morphine injection. PD_{Ant} is the diameter at the peak antagonist action in morphine-treated animals. Another indication of the antagonism is derived from the area under the time-effect curve (Figure 1b), according to the formula:

$$\frac{AUC_{Mo} - AUC_{Ant}}{AUC_{Mo}} \times 100$$

Where AUC_{Mo} and $(AUC_{Mo} - AUC_{Ant})$ refer to the curves following administration of morphine alone or morphine plus the antagonist, respectively. Log dose-response curves of the antagonists were plotted from the maximal inhibitory effects (MIE) of each treatment, ID_{50} values were estimated from the regression lines and 95% confidence intervals calculated by the usual method (Snedecor & Cochran, 1967). Separate groups of animals were also injected with the antagonists without morphine pretreatment.

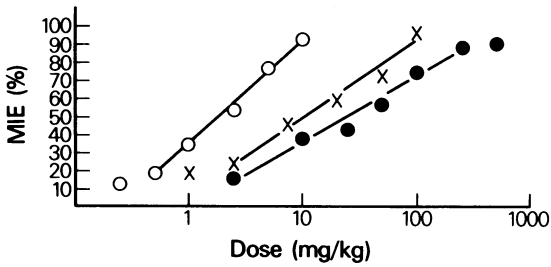

Figure 2 Dose-response curve of antagonism by nalorphine (●), levallorphan (×) and naloxone (○) to morphine-induced mydriasis in mice. The response is expressed in MIE (%) against dose of the antagonist (mg/kg).

Results

Morphine-induced mydriasis was inhibited by each of the antagonists, nalorphine, levallorphan and nalaxone in a dose-dependent manner (Figure 2). The inhibitory effect occurred within, and lasted for, several minutes (see below). The ID_{50} value (± s.e.) of nalorphine under the conditions of the study was found to be 26 ± 1 mg/kg.

The corresponding value for levallorphan was 11 ±

Table 1 Antagonism by nalorphine, levallorphan and naloxone to morphine-induced mydriasis

	Dose (mg/kg)	*MIE* (%)	*Reduction of AUC* (%)
Nalorphine	2.5	16	12
	10	38	22
	25	43	31
	50	57	50
	100	75	57
	250	89	85
	500	91	88
Levallorphan	1	18	12
	2.5	24	19
	7.5	44	38
	20	49	42
	50	71	61
	100	96	92
Naloxone	0.25	13	3
	0.5	19	7
	1	35	10
	2.5	44	22
	5	71	30
	10	93	77

The peak effects of the antagonists are expressed as their MIE, and the cumulative effect by reduction of AUC (see Methods).

2 mg/kg and for naloxone 2.0 ± 0.1 mg/kg. Thus the relative antagonistic potencies were 10: 56: 134, for nalorphine, levallorphan and naloxone respectively.

The AUC reduction and MIE were quantitatively similar for nalorphine and levallorphan, whereas for naloxone the MIE was significantly greater than the AUC reduction (Table 1), probably reflecting the shorter action of naloxone. The $T_{1/2}$ values, calculated from the AUC, were as follows: nalorphine, 173 min; levallorphan, 195 min; naloxone, 60 min. The respective ID_{50} values were 47 ± 3, 16 ± 5 and 7 ± 1 mg/kg (± s.e.).

Injection of maximal doses of levallorphan, naloxone or saline without morphine pretreatment did not result in any change of pupil diameters. Nalorphine caused a slight mydriasis, dilating the pupils by a maximum of 40%. When the highest dose (500 mg/kg) was used the half life of this response was 110 min.

Discussion

In the present work, we have determined the interaction of morphine and three narcotic antagonists: nalorphine, levallorphan and naloxone, on the diameters of the pupils in mice. The test dose of morphine used, 100 mg/kg, was chosen because it was found to produce maximal mydriasis. The inclusion of control groups injected with morphine alone enabled us to monitor the reproducibility of the opiate effect. Furthermore, we have measured the morphine effect in each animal prior to the injection of the antagonists.

Our results demonstrate that all three drugs produce a dose-dependent inhibition of morphine-induced mydriasis (Figure 2). This finding substantiates the view that the mydriasis produced by morphine in mice is a specific effect of narcotics (Korczyn *et al.*, 1979; Adler, Korczyn & Keren, 1980).

In order to characterize the antagonist activity, we have examined both the intensity of action and its duration.

The relative potencies of the antagonists calculated from ID_{50} values, were found to be similar to those given in the literature for various tests (e.g., Blane & Dugdall, 1968, Aceto, McKean & Pearl, 1969). The duration of action of the antagonists on the pupil also correlates well with the known properties of those drugs; nalorphine and levallorphan have similar duration of action of approximately 4 h (Jaffe & Martin 1975), while the action of naloxone is shorter (Blumberg & Dayton, 1974).

Nalorphine is known to have a weak agonistic activity, when injected alone (Kosterlitz & Watt, 1968). Our results, showing that nalorphine (but not the other antagonists) produced slight mydriasis, are consistent with this finding. In addition, the lack of effect of naloxone or levallorphan when injected alone suggests that endogenous enkephalins are not involved in the regulation of pupil diameter.

This method of measuring responses of the pupil to narcotic agonists and antagonists in mice has several advantages compared to standard methods used for determining opiate agonist or antagonist effect: (1) the method is simple, rapid, inexpensive, and can be repeated in every laboratory; (2) measurements of pupil diameter are accurate and minute changes can be detected; (3) unlike other techniques, this method is innocuous to the animal, and mice can be observed repeatedly over long periods without significant influence by physical change or stress (cf. e.g. the hot plate test where repeated examinations of the same animal are worthless); (4) Janssen & Jageneau (1956) have demonstrated that the mydriatic effect of opiates in mice correlates well with their analgesic effect.

The narcotic antagonists we tested, correlate in their potency and duration of action on the pupil in mice to their potency and duration of action as narcotic antagonists in man (Foldes, Lunn, Moore & Brown, 1963; Jasinski, Martin & Haertzen, 1967). Thus, the results obtained by this method allow prediction of other central effects in man.

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References

ACETO, M.D., McKEAN, D.B. & PEARL, J. (1969). Effects of opiates and opiate antagonists on the Straub tail reaction in mice. *Br. J. Pharmac.*, **36**, 225–239.

ADLER, C., KORCZYN, A.D. & KEREN, O. (1980). Tolerance to the mydriatic effects of morphine in mice. *J. neural Transm.*, **48**, 43–47.

BLANE, G.E. & DUGDALL, D. (1968). Interactions of narcotic antagonists and antagonist–analgesics *J. Pharm. Pharmac.*, **20**, 547–552.

BLUMBERG, H. & DAYTON, H.B. (1974). Naloxone, naltrexone and related noroxymorphines. In *Narcotic Antagonists, Adv. Biochem. Psychopharmac.* Vol. 8, ed. Braude, M.C., Harris, L.S., May, E.L. & Villareal, J.P. pp. 33–43. New York: Raven Press.

FOLDES, F.F., LUN, J.N., MOORE, J. & BROWN, J.M. (1963). N-allylmoroxymorphine: a new potent narcotic antagonist. *Am. J. med. Sci.*, **245**, 23–30.

FRASER, H.F., NOHS, T.L., VANHORN, G.D. & ISBELL, H.

(1954). Use of miotic effect in evaluating analgesic drugs in man. *Archs. Int. Pharmacodyn. Ther.*, **98**, 443–451.

JAFFE, J.H. & MARTIN, W.R. (1975). Narcotic analgesics and antagonists. In: *The Pharmacological Basis of Therapeutics*. ed. Goodman, L.S. & Gilman, A. 5th edition. pp. 245–283. New York: MacMillan.

JANSSEN, P.A.J. & JAGENEAU, A. (1956). Mydriatic activity of analgesics in mice. *Experientia*, **12**, 293–294.

JASINSKI, D.R., MARTIN, W.R. & HAERTZEN, C.A. (1967). The human pharmacology and abuse potential of N-allylnoroxymorphine (naloxone). *J. Pharmac. exp. Ther.*, **157**, 420–426.

KLEMFUSS, H., TALLARIDA, R.J., ADLER, C.H. & ADLER, M.W. (1978). Morphine-induced mydriasis and fluctuations in the rat; time and dose relationships. *J. Pharmac. exp. Ther.*, **208**, 91–95.

KORCZYN, A.D., BOYMAN, R. & SHIFTER, L. (1979). Morphine mydriasis in mice. *Life Sci.*, **24**, 1667–1674.

KOSTERLITZ, H.W. & WATT, A.J. (1968). Kinetic parameters of narcotic agonists and antagonists. *Br. J. Pharmac.*, **33**, 276–277.

SNEDECOR, G.W. & COCHRAN, W.G. (1967). *Statistical Methods*. 6th edition. Ames, Iowa: Iowa State University Press.

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