effect of ENK. However, Dobbins et al (1982) do not address the issue of whether another mediator, such as the secretagogue vasoactive intestinal polypeptide, (VIP), might be released by ACh. VIP is present in the mucosa/submucosa of the rabbit ileum, where it is released by electrical field stimulation (Gaginella et al 1981), and cholinergic-VIPergic interactions are possible within the neural plexuses of the gut (Gaginella & O'Dorisio 1979).

A direct effect of opiates on the intestinal epithelial cells cannot be completely ignored. However, in separate studies we have been unable to detect specific binding sites (receptors) for opiate ligands on membranes of isolated rat ileal and colonic epithelial cells (Gaginella et al 1983).

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Rapid induction of morphine dependence in the mouse by means of a modified pellet implantation

P. B. St. Leger*, N. A. Armstrong, P. S. J. Spencer, *The Welsh School of Pharmacy, UWIST, Cardiff CF1 3NU, U.K.*

The development of tolerance to and dependence upon the opiates is thought to depend upon the dose and frequency of drug administration. The ability to achieve persistent concentrations of opiates in target tissues during the processes of change and adaptation which underlie tolerance and physical dependence, is thought to be essential. The wide variation in the degree of tolerance to and dependence on opiates in the literature may be explained primarily by the many different methods used and their varying success in ensuring continuing exposure to the opiate during the preparative period. Many laboratory methods have been tested, ranging in complexity from simple but frequent multiple injections of opiate over days or weeks, to the implantation of osmotic mini-pumps (Gianutsos et al 1974; Wei & Loh 1976).

One of the most widely used methods of producing tolerance or dependence (or both) to morphine in laboratory animals is that of implantation of a tablet or pellet. First described by Maggiolo & Huidobro (1961), the pellet has been used extensively by Way et al (e.g. 1968, 1969) who used a pellet formulation described by Gibson & Tingstad (1970). Whilst we found this formulation satisfactory for inducing tolerance and dependence in rats, it was not so in mice. In successive experiments in mice, pellet implantation was followed by death within a few hours in 40 to 85% of animals, suggesting too rapid a release of morphine. Similar problems with different strains of mice have been reported by Brase et al (1977) and have led to the production of alternative 'pellets' (Hui & Roberts 1975). Some investigators have used a silicone-based delivery system, thereby avoiding tableting procedures (Isom et al 1978; Riffee et al 1980).

We describe modifications to the method of formulation equally useful in both mouse and rat studies.

Materials and methods

The following powders were used as received: anhydrous morphine base (Macfarlane Smith Ltd), microcrystalline cellulose (Avicel, Honeywell & Stein, Ltd),

* Correspondence.

Table 1. Comparison of formulae for morphine pellets.

Formula according to Gibson & Tingstad 1970	
Morphine base	75 mg
Microcrystalline cellulose	75 mg-filler
Calcium stearate	0·75 mg } glidants
Fumed silicon dioxide	1·5 mg }
Total pellet weight	152·25 ± 0·5 mg
Strength (hardness)	12 Strong Cobb Units
Dimensions	7 mm diameter
	3 mm thickness
Modified formula	
Morphine base	75 mg
Calcium phosphate	63·75mg } fillers
Sodium chloride	11·25 mg }
Calcium stearate	1.5 mg } glidants
Fumed silicon dioxide	1.5 mg }
Gelatin	6·65 mg—granulating agent
Total pellet weight	159·65 ± 0·5 mg
Strength (hardness)	2·5–3·0 kg
Dimensions	7 mm diameter
	3 mm thickness

Table 2. The effect of pellet crushing strength on the percent mortality in mice at different time intervals after implantation.

	% mortality*			
	24	48	72	96
Strength (kg)	Time after implantation (h)			
7	55	65	75	75
12	40	50	70	70
>20	15	25	70	70

* From groups of not less than 25 mice.

Table 3. The effect of different ratios of sodium chloride and calcium phosphate incorporated into the morphine-pellet formulation, on the mortality and naloxone-precipitated jumping response in mice.

NaCl: $CaPO_4$ ratio	% Mortality*	% Jumping
10 : 90	0	0
15 : 85	12	95 (of survivors)
25 : 75	70	100 (of survivors)

* From groups of not less than 25 mice.

fumed silicon dioxide (Aerosil, K & K Greeff, Ltd), calcium phosphate (Allbright & Wilson, Ltd.), calcium stearate, sodium chloride, gelatin and lactose (BDH Ltd) and light magnesium carbonate (Macarthys).

Preparation of granules. The formulae for the original and new morphine pellets are given in Table 1. In our preliminary work we used the formula and method described by Gibson & Tingstad (1970), except that the amount of silicon dioxide used was doubled to improve powder flow during tableting. Placebo pellets were made by the same process, substituting an equivalent amount of lactose for the morphine base. Two 'wet-granulation' methods were also examined for the 'original' formulation. In the first, morphine base and microcrystalline cellulose were granulated together, using a 10% w/v solution of gelatin, the damp mix then being screened through a 2 mm mesh sieve; the resultant granules were dried in a hot air oven at 60 °C for 1 h. Finely screened silicon dioxide and calcium stearate were added to the dried granules, the whole mixed and screened again, this time through a 710 μm mesh sieve. The alternative granulation process involved the wet granulation of morphine base alone, the microcrystalline cellulose being added (with silicon dioxide and calcium stearate) to the dried granules.

For pellets of the new formula, the wet granulation procedure was used, the morphine base, calcium phosphate and sodium chloride being granulated together with 10% w/v solution of gelatin. Sieving, drying and the final preparation of granules was conducted as before. Placebo pellets contained 75 mg of light magnesium carbonate in place of the morphine base.

Compression of pellets. The theoretical pellet weight, allowing for added gelatin, was calculated for each batch of pellets. The dry granules were compressed using an eccentric tablet press (Manesty Machines Ltd., model E2), fitted with 7 mm concave punches. Pellet crushing strength was set at 2·5 to 3·0 kg, a strength equivalent to that used by Gibson & Tingstad (1970), unless pellets of greater strength were prepared deliberately (see Table 2). Once the correct pellet weight and strength had been adjusted, the tablet machine was set to these parameters and the batch of pellets run off. Periodic checks of pellet weight and strength were made during the batch run. Pellet strength was determined using a Monsanto hardness meter.

Animals, husbandry and drug administration. Male GB1 mice, 18 to 22 g, were housed in groups of 5 and maintained at 21 ± 1 °C in a 12 h (normal phase) light-dark cycle. They were fed a conventional 41B cube diet and had free access to tap water, food and water being withdrawn 2 h before pellet implantation or before naloxone challenge.

Implantation was the subcutaneous insertion of a single pellet containing 75 mg morphine base under the dorsal skin in the scapular region of the mouse, under light ether anaesthesia. Recovery from the anaesthetic was almost complete at 10 min, when mice were returned to their home cages. Evidence of morphine dependence was elicited by precipitating a withdrawal syndrome, using a single intraperitoneal injection of naloxone injected 72 h after morphine implantation, unless otherwise stated. The intensity of morphine withdrawal was illustrated by the number and frequency of stereotyped jumping episodes during the ensuing 15 min, using a photoelectric digital counter (St. Leger 1982).

Results and discussion

Changes in pellet strength. Three batches of granules were manufactured to the original formula, with compression varied to provide pellets of 7, 12 and >20 kg crushing strength. Comparison of the results obtained when these pellets were implanted into mice is shown in Table 2. Results after 24 h showed that the >20 kg strength pellets produced far fewer deaths than the two other compressions, but by 72 h after implantation this improvement had disappeared, leaving no persistent difference between the mortality in the three groups. Increasing the strength of the pellet simply delayed the death of the mice by 24–48h.

Changes in method of formulation. A wet-granulation technique, either for the morphine base and microcrystalline cellulose combined, or for the morphine base alone, had no effect on subsequent mortality. Postmortem of the implantation sites revealed that the morphine pellets had disintegrated in-situ, resulting in a far greater surface area from which absorption of morphine could occur. This observation is in direct contrast to the report by Gibson & Tingstad (1970) although Riffee et al (1980)—using the same formulation—found that the pellet became soft and difficult to remove after implantation while Cochin et al (1979) wrapped pellets in small nylon bags before implantation to facilitate their subsequent removal. That the pellets tended to disintegrate was not surprising since microcrystalline cellulose may be used as a tablet disintegrant.

Pellets made by the new formulation. Since the Gibson & Tingstad (1970) pellet did not exhibit the physical properties claimed for it, the formulation was changed to produce a pellet that once implanted would retain its form, whilst slowly releasing morphine over a period of days. Initially, calcium phosphate was selected to replace cellulose on the basis of its insoluble property, coupled with the fact that it could be compressed to make hard pellets. Experiments with pellets made from a formulation of just morphine and calcium phosphate showed that the pellets retained their integrity after implantation and animal mortality was prevented. Unfortunately, naloxone was unable to precipitate withdrawal signs, even up to 7 days after implantation. This suggested that release of morphine from the insoluble pellet matrix was too slow. The problem was solved by incorporating a small amount of sodium chloride into the formulation. On implantation, this dissolves, opening up small channels into the pellet through which morphine diffuses. Preliminary experimentation determined a suitable amount to be 15% (Table 3), at which concentration mortality was less than 15%, and of survivors 95% showed withdrawal jumping.

Optimum implantation time for the new formula pellet was found to be 72–96 h, although jumping may be elicited by naloxone challenge as early as 24 h after implantation. Furthermore, the relationship between the intensity of jumping and the dose of naloxone challenge appeared to be linear over the range of doses studied. Jumping can be elicited by a challenge dose of 0·5 mg kg^{-1} naloxone at the optimum interval of 72 h post-implantation.

The optimum implantation time using the modified pellet proved to be similar to that reported by Way et al (1969) for the Gibson & Tingstad (1970) formulation, and also similar to times reported for the silicone based delivery systems (Isom et al 1978; Riffee et al 1980). Only Isom et al (1978) have attempted to measure blood concentrations of morphine associated with their implant and they report that peak blood values occurred 12 h after implantation and these declined slowly over the next 144 h. Subsequent behavioural experiments have confirmed the initial observations in mice and extended them to rats and we conclude that this formulation provides a reliable method for the rapid induction of tolerance to and dependence on morphine.

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