Brain and plasma concentrations of morphine during the development of physical dependence and tolerance

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The subcutaneous implantation of a morphine pellet has been used to produce physical dependence and tolerance to morphine in mice (Maggiolo & Huidobro, 1961; Way, Loh & Shen, 1969) and rats (Cicero & Meyer, 1973). The degree of physical dependence and tolerance which develops in mice (Way & others, 1969) and rats (Cicero & Meyer, 1973) is comparable to that produced by an injection schedule of morphine. Recently, Patrick, Dewey & others (1975) reported that the concentration of morphine in the mouse brain increases rapidly and is maintained at a high level through the third day following morphine pellet implantation. This study was initiated in an effort to further quantify the technique of pellet implantation in the rat so that this model could be used for additional studies in our laboratory.

Male, Sprague-Dawley derived rats (Laboratory Supply Co., Indianapolis, IN) six to eight weeks old (180–200 g) were employed. Food and water were freely available and animals were housed in community cages unless otherwise noted. Temperature was maintained between 21–23° and a 14 h light cycle (0600 to 2000 h) was employed.

Two morphine pellets, each containing 103·5 mg morphine base and formulated according to the procedure of Gibson & Tingstad (1970), were implanted subcutaneously 2–4 cm posterior to the shoulders on the back of the animals under light ether anaesthesia. The mortality following implantation of two morphine pellets was between 3–5% for all experiments provided that the animals were housed individually for at least 24 h following pellet implantation. Morphine pellets remained implanted in the rats during all experimental procedures except where noted.

The degree of physical dependence on morphine was determined using a modification of the procedure of Cicero & Meyer (1973). Acute morphine withdrawal was precipitated by the administration of naloxone hydrochloride (0·4 mg kg^{-1}, s.c.) and the number of 'wet dog' shakes were counted for a period of 30 min (a shorter period than that used by Cicero & Meyer but the results of the test are not affected). Symptoms of the withdrawal syndrome included writhing, salivation, lacrimation, teeth chattering, profuse diarrhoea, ejaculate-like discharge, hostility, bursts of activity which included escape behaviour and 'wet dog' shakes. Physical dependence, as determined by the number of 'wet dog' shakes, reached the highest level four to five days after implantation of 207 mg morphine base (ave. of 29 shakes per rat in 30 min on both days four and five). The number of 'wet dog' shakes per rat in 30 min declined slowly over the next six days but was still approximately 20 shakes per rat on day 11. However, on days 9 and 11, the rats were demonstrating withdrawal behaviour (hostile to touch, fighting, and 'wet dog' shakes) before naloxone injection. It should be noted that Cicero & Meyer (1973) reported that the number of 'wet dog' shakes peaked three days after implantation of 75 mg of morphine base. This apparent discrepancy may best be explained by previous investigations in mice which have shown that the degree of physical dependence and tolerance is determined by the amount of morphine administered (207 mg in the current study) and the interval between doses and not on the duration of administration (Goldstein & Sheehan, 1969).

Tolerance development to the lethal effect of morphine was determined 2, 3, 4, 5, and 7 days following morphine pellet implantation. Animals were housed individually and mortality was determined 24 h after acute morphine administration. LD50 determinations were calculated utilizing the Reed-Meunch method as described by Miya, Holck & others (1973). The 24 h LD50 of morphine in naive control animals was 216 ± 14 mg kg^{-1}. Two days following implantation of 207 mg of morphine base all animals survived doses of morphine base up to 570 mg kg^{-1}. On days 4 and 5 following morphine implantation, LD50 values were calculated to be 1750 ± 95 mg kg^{-1} and 1500 ± 114 mg kg^{-1} respectively. On day 7, two rats died at the highest dose of morphine administered (1250 mg kg^{-1}). Thus, it appears that the time course of tolerance development to the lethal effect of morphine closely parallels that of physical dependence development.

Morphine concentrations in plasma and brain tissue were assayed utilizing a commercial radioimmunoassay kit (Abuscreen, Roche Diagnostics) which is a modification of the procedure described by Spector & Parker (1970). To prepare samples for morphine determination, plasma was diluted (1 to 10 or 1 to 20) and brain tissue was homogenized (1 to 4 or 1 to 5) in normal saline. These dilutions were made so that the concentration of morphine in 1·0 ml of sample would fall in the range of the control standard curve (15 to 90 ng ml^{-1} morphine). The plasma concentration of morphine (Fig. 1) rises slowly reaching the highest level (1585 ng ml^{-1}) five days after pellet implantation and then declines such that on day 11 only a small amount of morphine remains (169 ng ml^{-1}). Brain concentrations of morphine peak quickly and are maintained for seven days following pellet implantation (Fig. 1). As with plasma concentrations, brain morphine concentrations decline significantly after day 7. The data suggest that, in this model, morphine physical dependence and tolerance are related

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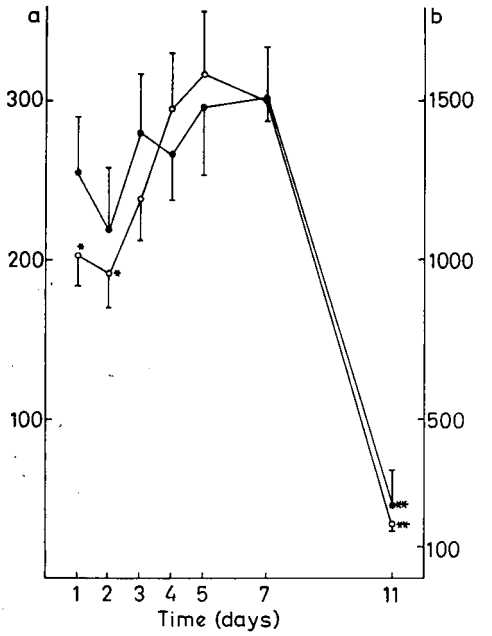

FIG. 1. Brain (a) and plasma (b) morphine concentrations (ng g^{-1} ± s.e.) during dependence development. ●—● Brain and ○—○ plasma morphine concentrations were measured during development of morphine dependence using a radioimmunoassay technique. Each point represents the mean of at least 4 rats. * Significantly different from day five (plasma) at $P < 0.05$. ** Significantly different from all other points at $P < 0.05$. One-way analysis of variance and Neuman-Keuls were utilized in making comparisons between means.

to the concentration of morphine found in the plasma and brain.

In an effort to ascertain the amount of morphine absorbed, pellets were removed and assayed for morphine 2·5, 5, 7·5, and 10 days after implantation using a differential absorbance method (Wahbi & Farghaly, 1970) after extraction of morphine by a modification of the method of Kupferberg, Burkhalter & Way (1964). This method is highly reproducible and has a sensitivity of approximately 1 mg of morphine per pellet granuloma.

It was noted that the pellet disintegrates within two days after implantation and along with apparent vascularization of the granuloma, allows for absorption of morphine. The fibrous granuloma, along with any accumulated pus-like fluid, was included in the assay. The absorption of morphine from the pellet granuloma appears to be linear ($r = 0.959$) with 85% of the morphine absorbed 7·5 days following implantation. By day 10, only a small amount of morphine remains (4 mg per granuloma) explaining the spontaneous withdrawal symptoms noted in the previously described experiment. The data indicate complete absorption of morphine from the implantation site at a rate of approximately 23 mg day^{-1} resulting in a daily dose of 90 mg kg^{-1} in this experiment.

Way & others (1969) found that absorption of morphine from pellets was incomplete. It has been suggested that such incomplete absorption might be due to encapsulation of the pellets by fibrous tissue (Bläsig, Herz & others, 1973) or the cellulose in the formulation blocks further absorption after the morphine at the surface of the pellet is absorbed (Gibson & Tingstad, 1970). However, Patrick, Dewey & others (1975) reported that encapsulation of the morphine pellet in mice did not cause an appreciable decrease in morphine absorption. The complete and linear absorption of morphine from the pellets noted in the present study, although different from that reported by Bläsig & others (1973), may be due to the fact that the pellets disintegrate after implantation which would greatly increase the area of absorption. This allows a relatively constant brain concentration of morphine until the pellet is completely absorbed.

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