Quantitation of morphine tolerance induced by pellet implantation in the rat

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Five schedules of subcutaneous morphine pellet implantation were developed to induce morphine tolerance in the rat. These differed in the number of pellets used and the interval between implantations. Two pellets, each containing 75 mg of morphine base, were implanted over a 3-day period (schedule 1), four morphine pellets over a 3-day period (schedule 2), six pellets over a 3-day period (schedule 3), six pellets over a 7-day period (schedule 4), and 10 pellets over a 10-day period (schedule 5). The degree of morphine tolerance was quantitated in rats from each schedule by determining the median analgesic dose (AD50) of morphine sulphate. The AD50 values increased from 3·6 to 53·1 mg kg^{-1} in schedule 5 rats (a 15-fold tolerance), whereas, only a three fold tolerance was produced in schedule 1 rats. Rats in schedules 2 to 4, developed six, eight and 13-fold tolerance to morphine, respectively. Rats that were less tolerant to morphine had lower brain and plasma concentrations of morphine at the time of pellet removal and also faster disappearance of morphine from brain and plasma compared with rats that were more tolerant to morphine. The data suggest that implantation of two morphine pellets produces a low degree of tolerance and that caution should be exercised when comparisons are based on findings from rats or mice the biochemical data when four or less number of morphine pellets are used to induce tolerance.

Morphine pellet implantation has been used to induce tolerance to, and physical dependence, on morphine in mice (Way, Loh & Shen, 1969; Hui & Roberts, 1975) and in rats (Bläsig, Herz & others, 1973; Cicero & Meyer, 1973; Wei, Loh & Way, 1973). The degree of tolerance to, and physical dependence on morphine has been quantitated in mice (Way & others, 1969). But in rats most investigators have implanted one or two pellets, each containing 75 mg of morphine base, for a 3-day period and assumed that a sufficient degree of tolerance and physical dependence developed. However only a two to three fold tolerance in rats resulted from implantation of two morphine pellets for three days (Bhargava, Afifi & Way, 1973).

Ten pellets, each containing 75 mg morphine base, over 10 days produced a high degree of dependence, whereas four pellets over 3 days produced a mild degree of dependence (Bhargava, 1977). Markedly different or contradictory conclusions have been reached when different methods were used to induce morphine tolerance and dependence.

For example, Shen, Loh & Way (1970) showed that in mice rendered dependent on morphine by pellet implantation, brain 5-HT turnover was increased. In contrast, Marshall & Grahame-Smith (1971) failed to show increases in brain 5-HT turnover in mice rendered dependent by the multiple injection technique. Similarly, Algeri & Costa (1971), implanted two pellets containing 75 mg of morphine base in rats, 70 h apart and found no change in brain 5-HT turnover, but they did observe some signs of withdrawal precipitated by high doses of nalorphine although they did not provide a quantitative measure of either the degree of tolerance or dependence on morphine.

Five schedules of morphine pellet implantation for rapid induction of morphine tolerance in rats are now described. The degree of tolerance developed has been assessed quantitatively using the classical tail-flick procedure. The brain and plasma concentration of morphine at various times after pellet removal have also been determined.

Male Sprague-Dawley rats (Locke Erikson Lab., Inc., Melrose Park, Illinois), 150–200 g, were housed three to a cage, with food and water continuously available, and maintained on a 12 h dark–light cycle (lighted 06.00–18.00) in a room with controlled temperature (23 ● 1°) and humidity (65 ± 2%).

The rats were rendered tolerant to morphine by five different schedules utilizing subcutaneous implantation in the lower back under light ether anaethesia of specially formulated morphine pellets (Gibson & Tingstad, 1970), each containing 75 mg of morphine base. The control rats received pellets containing the excipients but no drug. In schedule 1, one pellet was implanted on day 1, the second was implanted 1·5 days later. Both were removed under light ether anaesthesia three days after the first implantation. In schedule 2, two pellets were implanted on the first day and two more 1·5 days later. All were removed 1·5 days after the implantation of the third and fourth pellet. In schedule 3, one pellet was implanted on the first day, two on the second day, and three on the third day. All were removed on the fourth day. In schedule 4, one pellet was implanted on the first day, two on the third day, and three on the fifth day. All were removed on the eighth day. In schedule 5, one pellet was implanted on the first day, two on the third day, three on the fifth day, and four on the eighth day. All were removed on the tenth day. Controls were treated similarly.

After pellet removal, sufficient time (7–8 h) was allowed for the tail-flick reaction time, estimated according to Way & others (1969), to return to pre-

implantation control values of 4·0 ± 0·4 s (s.e.) and when the mean base line response had been measured the tail-flick reaction time was redetermined 30 min after subcutaneous injection of morphine sulphate. A reaction time of eight or more seconds was considered to be a positive analgesic response. A quantal response for three doses of drug was determined. Eight to 10 rats were used for each of the three doses. Dose-response curves were drawn by linear regression analysis. The median analgesic dose (AD50) of morphine sulphate, the potency ratio and their 95% confidence limits were determined by the method of Litchfield & Wilcoxon (1949).

Morphine pellets were removed from the rats which at various times thereafter were decapitated and the blood collected in heparinized tubes and brains rapidly frozen on dry ice. Plasma was separated by centrifugation in a refrigerated centrifuge and frozen. Brain and plasma samples were analysed for morphine by the fluorometric method of Kupferberg, Burkhalter & Way (1964).

Brain concentrations of morphine in rats declined with time after the morphine pellets were removed (Table 1).

The rate of elimination of morphine from brain and plasma was faster in schedule 1 animals compared with schedule 5 rats. The half life of morphine in brain of schedule 5 rats was found to be 3·8 h.

In rats from schedules other than 1, 2 and 5, most of the morphine disappared from the brain and plasma, 8 h after pellet removal. Rats in schedule 5 had low but detectable concentrations of morphine in brain and plasma 24 h after pellet removal.

Table 1. *Morphine concentration in brain and plasma of morphine dependent rats from various schedules of pellet implantation.*

Schedule	Pellets	Days	Time after pellet removal h	n	Morphine concn ng g^{-1} or ng ml^{-1} ± s.e. Brain	Plasma
1	2	3	0	4	230 ± 5	510 ± 10
			4	5	—	—
2	4	3	0	4	530 ± 40	980 ± 40
			4	5	20 ± 10	80 ± 40
			6	4	—	—
3	6	3	0	4	560 ± 30	1040 ± 30
			4	5	270 ± 10	510 ± 20
			6	5	40 ± 2	90 ± 10
			8	4	—	—
4	6	7	0	4	1360 ± 40	2570 ± 80
			4	5	540 ± 20	940 ± 70
			6	5	250 ± 10	540 ± 10
			8	5	—	—
5	10	10	0	4	1500 ± 30	2850 ± 10
			4	5	700 ± 50	1570 ± 40
			6	5	300 ± 10	880 ± 60
			8	5	30 ± 10	100 ± 30
			24	5	20 ± 10	40 ± 10

Dashes indicate concentrations below the lowest detectable concentration.

Table 2. *Effect of morphine pellet implantation on development of tolerance to morphine in the rat.*

Schedule[a]	Pellets	Days	Morphine sulphate AD50, mg kg^{-1} (95% limits)	Potency ratio[c] (95% limits)
Control[b]	—	—	3·58 (1·54–8·30)	—
1	2	3	11·00 (7·33–16·50)	3·08 (1·21–7·85)
2	4	3	23·33 (6·64–81·99)	6·53 (1·92–22·19)
3	6	3	28·81 (13·05–63·62)	8·06 (3·02–21·52)
4	6	7	46·67 (22·94–94·91)	13·03 (4·42–38·44)
5	10	10	53·13 (29·03–97·23)	14·84 (5·30–41·55)

[a] Rats were rendered tolerant to morphine by subcutaneous implantation of morphine pellets as described in the text. Morphine sulphate AD50 was determined in rats from all five schedules of morphine pellet implanted rats and control pellet implanted schedules 1 and 5 rats.

[b] Morphine sulphate AD50's in control implanted schedules 1 and 5 rats were identical.

[c] Comparisons were made with AD50 in control pellet implanted rats.

Rats on all five schedules, developed tolerance to morphine as evidenced by increases in the dose required to produce analgesia (Table 2). Eight h after the pellet removal, the tail-flick reaction times had returned to pre-implantation reaction time and were identical to those in controls even in schedule 5 animals in which morphine could be detected in the brains. The degree of tolerance developed as evidenced by the ratio of morphine sulphate AD50 of tolerant over that of non-tolerant rats was 3-fold and 15-fold in schedules 1 and 5 treated rats, respectively (Table 2). Similarly, a 6·5, 8 and 13-fold tolerance to morphine developed in schedules 2, 3 and 4 animals, respectively.

A linear relation existed between the morphine sulphate AD50 and the morphine concentration in brain or plasma immediately after pellet removal. The regression correlation coefficient r = 0·993 showing a highly significant fit of the data to the linear relation

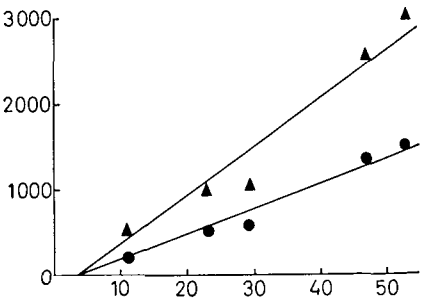

FIG. 1. Correlation between the brain and plasma concentration immediately after pellet removal and morphine sulphate AD50. The five points represent the five schedules used. ●—Brain, r = 0·987, ▲—plasma, r = 0·993. Ordinate: Morphine concentrations at 0 h after pellet removal (ng g^{-1} or ng ml^{-1}). Abscissa: Morphine sulphate AD50 (mg kg^{-1}).

between morphine AD50 and its plasma concentration. Similarly, r = 0·987 for the linear relation between morphine sulphate AD50 and brain concentration of morphine (Fig. 1). Thus, as the higher morphine concentration in brain or plasma was maintained, the degree of tolerance development also increased. When the regression lines were extrapolated to zero morphine concentration in brain and plasma, the equivalent to control pellet implantation, the morphine AD50 was 3·5 mg kg^{-1}, a value in agreement with that in control pellet implanted rats (3·58 mg kg^{-1}).

Thus in rats, it is possible to produce a high degree of tolerance to morphine in a short time by subcutaneous implantation of morphine pellets. Of the five schedules used, schedule 1 produced the lowest, while schedule 5 produced the highest degree of tolerance.

The development of degree of tolerance was directly related to the number of morphine pellets implanted and to the duration of the implantation period. The results for schedule 1 confirm our previous finding that the implantation of two pellets, each with 75 mg of morphine base, 1·5 days apart, produces only a three-fold tolerance to morphine in rats (Bhargava & others, 1973). This is in contrast to the ten to 15-fold tolerance that develops in mice after implanting one morphine pellet for three days (Bhargava & Way, 1972). The present study shows that to induce a degree of tolerance in the rat comparable to that produced by one pellet in mice, at least ten pellets should be implanted for 10 days.

The degree of tolerance produced in rats by pellet implantation was dependent on the concentration of morphine in brain and plasma at the time of pellet removal. A linear relation was found to exist between morphine sulphate AD50 of rats from various schedules and their brain and plasma concentration. The low degrees of tolerance and dependence that develop when only one or two pellets of morphine are used are associated with the low concentrations of drug in brain and blood.

The present study indicates that in assessing the biochemical mechanisms of morphine tolerance it is essential to quantitatively assess the degree of tolerance to morphine before inter- and intra-species comparisons are made.

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