Pharmacology Biochemistry & Behavior, Vol. 5, pp. 603–607.

A Comparison of Withdrawal in Rats Implanted With Different Types of Morphine Pellets[1]

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(Received 19 May 1976)

MEYER, D. R. AND S. B. SPARBER. *A comparison of withdrawal in rats implanted with different types of morphine pellets.* PHARMAC. BIOCHEM. BEHAV. 5(6) 603–607, 1976. –Time course and duration of physical dependence was investigated in rats implanted subcutaneously with 3 different types of morphine (M) pellets. Each was formulated according to the method of Gibson and Tingstad [8], but differed in surface area and hardness. Animals were maintained for 19 days after implantation and physical dependence was assessed every other day. Severity of naloxone (Nx)-induced withdrawal was quantified by the use of a composite symptom score and weight loss. Withdrawal severity was greatest following implantation of a pellet (Type C) of large surface area and low hardness rating, and least following implantation of a pellet (Type A) of small surface area and high hardness rating. Abstinence severity which resulted from implantation of a pellet (Type B) of moderate surface area and low hardness rating was intermediate. When 2 pellets were implanted the difference between Type C and B was amplified. It was concluded that formulation per se was not sufficient for specifying M pellet characteristics.

Morphine pellets Naloxone Precipitated abstinence

SUBCUTANEOUS implantation of M pellets has been a conventional method used for the induction of physical dependence on opiates in mice [4, 10, 13, 14] and rats [3, 5, 15]. Even though the development of dependence is rapid [5,14], estimates of the duration and severity of physical dependence are variable. Attempts to standardize the procedure by using M pellets manufactured from the same formulation have not been entirely successful. Some laboratories have reported that peak dependence subsides after 24–48 hr [5, 13, 14]. Other investigators have observed that constant dependence can last for as long as 3–6 days after pellet implantation [3, 9, 15]. More recently, Gellert and Sparber [7] have demonstrated that stable dependence could be maintained for as long as 11 days after implantation of a single M pellet. Since that publication, preliminary experiments in our laboratory indicate that variability between different batches of pellets may be correlated with differences in pellet surface area and hardness. As a result, the present experiment was designed to evaluate, in a more rigorous fashion, the degree of physical dependence that developed following implantation of 3 different types of M pellets. The 3 types chosen for comparison were representative of pellets which had been used in our laboratory for previous experimentation. All pellets were manufactured from the same formulation, but differed with respect to surface area and hardness.

METHOD

Animals and Chemicals

Male Sprague-Dawley rats (280–300 g) purchased from Bio Laboratories (White Bear Lake, MN) were used. The animals were housed in the laboratory for 2 weeks prior to use. All subjects were individually housed in air-conditioned quarters at 25°C and 50% humidity under a 12 hour lighting regimen (lights on from 0600 to 1800). Food and water were available in their home cages ad lib. All animals were morphine-naive. M pellets were formulated according to the procedure of Gibson and Tingstad [8]. Placebo pellets were also formulated in the same way except that an equivalent amount of lactose was substituted for M. Naloxone was donated by Endo Laboratories, Inc. (Garden City, NY). The specifications of the M and placebo pellets are shown in Table 1. As illustrated, all 3 M pellets differed, to some extent, in diameter and thickness. Total surface area varied from small to large (A < B < C). Hardness rating varied from low to high (A > B = C). In general, the physical characteristics of Pellet B were the most variable, while the characteristics of Pellet C were the most uniform. The thickness of each tablet was calculated as the difference between the minimal and maximal values. Minimal thickness represented the dimension along the edge and maximal thickness represented the dimension in the center

[1] This investigation was supported in part by grants MH 08565 and DA 00532 from the USPHS.
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TABLE 1

SPECIFICATIONS OF MORPHINE AND PLACEBO PELLETS

Pellet	Diameter (mm)	Thickness (mm)	Surface Area (cm^2)	Weight (mg)	Hardness (Strong Cobb Units)
A(8)*	7.06 ± .002†	2.76 ± 0.01	1.39 ± 0.01	151.5 ± 1.4	20.2 ± 1.2
B(8)	7.04 ± 0.02	3.22 ± 0.16	1.49 ± 0.02	157.5 ± 5.0	8.6 ± 1.4
C(5)	9.54 ± 0.02	2.75 ± 0.02	2.08 ± 0.02	153.9 ± 0.2	9.8 ± 0.4
D(7)	7.06 ± 0.02	2.66 ± 0.01	1.37 ± 0.01	150.7 ± 1.4	7.2 ± 0.6

A–C indicate the 3 types of M pellets used: A (50 mg morphine base): B and C (75 mg morphine base). D indicates the placebo pellet (0 mg morphine base).
*Number of pellets assessed.
†Mean ± SEM.

of each tablet. Mean total surface area was calculated using the formula: $2(\pi r^2) + 2\pi rh$, where r = radius and h = thickness. Hardness rating was measured in Strong-Cobb hardness units. The Strong-Cobb hardness tester measures the diametrically applied force required to break the tablet. The final breaking point is recorded in 30 arbitrary units. In general, hardness ratings as measured by this apparatus tend to be higher than those measured by other conventional machines [12].

One group of animals (N = 5) was implanted with a placebo pellet. Three groups of animals (N = 5 in each group) were implanted with a single M pellet (either A, B or C). These 3 groups will be designated as Pellet A_1, B_1 or C_1. Two additional groups of animals (N = 5 in each group) were implanted with two M pellets (either B or C). These 2 groups will be designated as Pellet B_2 or C_2. All 6 groups were implanted at the same time and used concurrently.

Implantation Procedure

The animals were anesthetized with sodium pentobarbital (30 mg/kg IP) and a 2 cm incision was made in the shoulder region. The pellets were inserted 1 to 2 cm from the incision under the skin and the wound was closed with clips.

Estimation of Physical Dependence

The development of physical dependence was assessed at alternating daily intervals (every other day) for 19 days after M or placebo pellet implantation (see below). The M or placebo pellets were not removed during the experiment. On Days 1, 3, 5, etc. each animal received a single injection of naloxone HCl (2.5 mg/kg, IP in normal saline). On Days 2, 4, 6 etc. each animal received a single injection of the vehicle (1 ml/kg, IP). Each animal was slightly sedated with carbon dioxide (CO_2) prior to injection of either naloxone or saline. This was acheived by having the animal inhale pure CO_2 for several seconds before injection. This procedure was used to facilitate handling of the animals and to minimize restraint stress during injections. The severity of precipitated abstinence was assessed for 15 min following the injection of either naloxone or saline. The following 11 abstinence symptoms were recorded: rhinorrhea, urination, diarrhea, ptosis, piloerection, teeth chattering, wet-dog shakes, irritability to handling, salivation, ejaculation, and lacrimation. These symptoms were counted as quantal events and assigned a score of either 0 or 1. A score of 0 was given if the symptom did not appear, and a score of 1 was given if the symptom appeared any time during the 15 min period. Scores for all of the symptoms were summed to provide a total or composite score representing precipitated abstinence severity for each animal. In addition, body weight was recorded at 0 min, 15 min, 1 hr, 2 hr, and 3 hr after either saline or naloxone injections. Body weight was monitored to the nearest gm by using a standard laboratory animal balance. Even though body weight was monitored at varying times after the naloxone challenge, the peak effect of the precipitated weight loss was evident 1 hr after the injection for groups implanted with M pellets. At that time, approximately 90% of the total drug effect was present. Since group differences in observed weight loss were independent of the measurement interval, only the data at 1 hr postinjection were analyzed. Loss in body weight 1 hr after naloxone or saline was used as a second index to characterize the severity of the precipitated abstinence syndrome.

The severity of the precipitated abstinence syndrome for placebo and M pellet(s) implanted animals was compared. To obtain quantitative indices of the precipitated M abstinence syndrome the area under the abstinence response-time curves for the symptom-profile and body weight loss were measured. Integration of the area under the curve was calculated by the trapazoid method utilizing 2 adjacent daily scores as the points from which a perpendicular line to the abscissa was drawn. These areas were summed to provide the total area under the curve for 1–19 days after M pellet implantation. Total areas under the response-time curves for each group were compared by analysis of variance.

RESULTS

The withdrawal syndrome which developed after the injection of naloxone in M implanted animals was consistent within groups implanted with the same pellet. The injection of naloxone in animals implanted with the placebo pellet elicited no measurable abstinence reaction. Saline injections in either placebo or M implanted animals were also ineffective in this regard.

The mean number of abstinence symptoms which appeared 15 min after the injection of naloxone in groups of animals which were implanted with a single M pellet (either A, B or C) differed dramatically (Fig. 1). The results of the single pellet implant indicated that peak physical

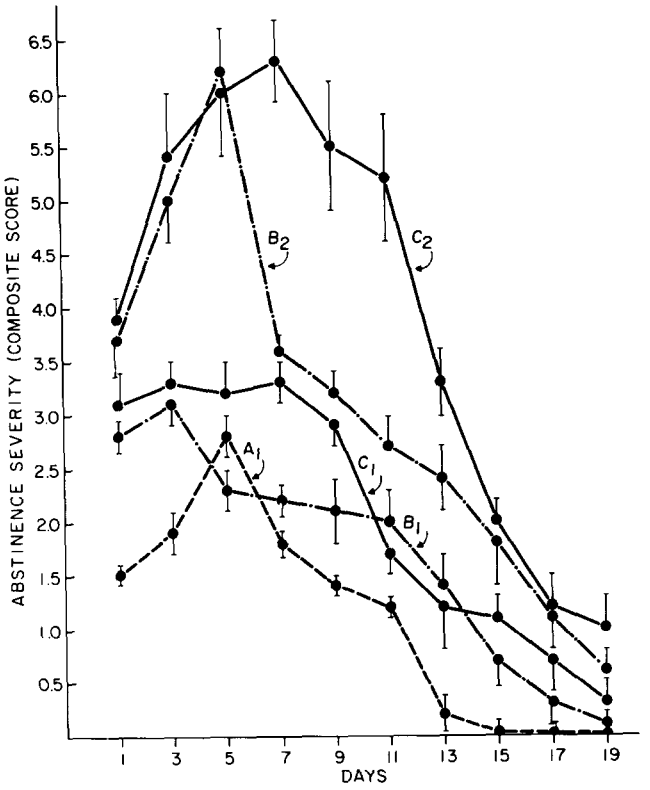

FIG. 1. Abstinence severity (symptom profile) induced by naloxone (2.5 mg/kg IP) 15 min after the injection for 5 groups of rats which were implanted (SC) with three different batches (A, B or C) of morphine pellets. Three groups of rats were implanted with a single morphine pellet from one of the three batches (A_1, B_1 or C_1). Two additional groups of rats were implanted with two morphine pellets from two of the batches (B_2 or C_2). The data represented indicate the mean ± SE for groups of 5 rats each. See text for an explanation of the specifications of each type of pellet. The abscissa indicates days after implantation.

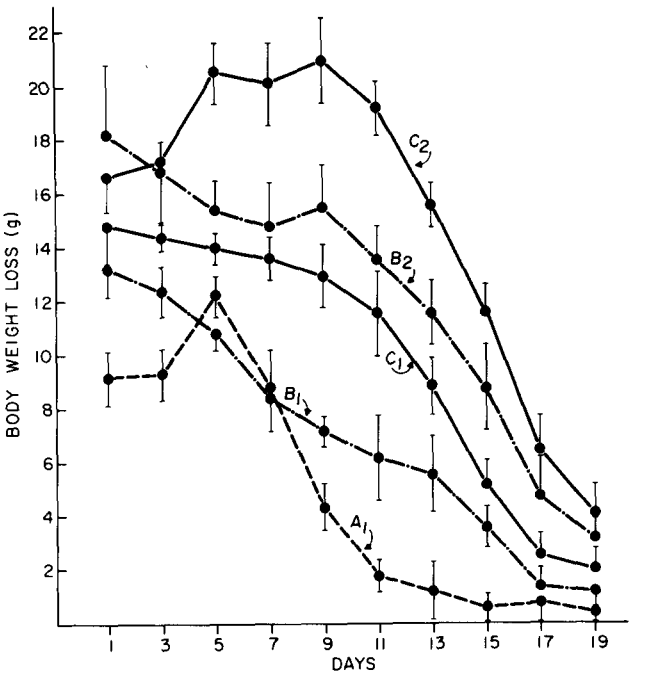

FIG. 2. Body weight loss induced by naloxone (2.5 mg/kg IP) 1 hr after the injection for 5 groups of rats which were implanted (SC) with three different batches (A, B or C) of morphine pellets. The data shown indicated the peak naloxone effect. For clarity, weight loss at the other measured time intervals, (i.e., 15 min, 2 and 3 hr after injection) were not shown. Three groups of rats were implanted with a single morphine pellet from one of the three batches (A_1, B_1 or C_1). Two additional groups of rats were implanted with two morphine pellets from two of the batches (B_2 or C_2). The data represented indicate the mean ± SE for groups of 5 rats each. See text for an explanation of the specifications of each type of pellet. The abscissa indicates days after implantation.

dependence developed quite rapidly in the animals that were implanted with either Pellet B or C, but not Pellet A. The withdrawal syndrome which developed after the injection of naloxone in the 2 former groups of animals appeared to have peaked by 24 hr after the pellet implantation. On the other hand, the severity of the withdrawal syndrome in the animals which were implanted with Pellet A did not show peak intensity until 5 days after pellet implantation.

As illustrated in Fig. 1 the total severity of the abstinence syndrome differed dramatically among the groups that were implanted with the 3 types of M pellets. An analysis of the total area under the curve indicated that all 3 groups differed in the extent of abstinence ($p < 0.05$). Animals implanted with Pellet C showed a greater total abstinence score than animals implanted with Pellet B or A ($p < 0.05$ and < 0.01, respectively), and animals implanted with Pellet B. showed a greater total abstinence score than animals implanted with Pellet A ($p < 0.05$). The activity of Pellet B remained high for 1–3 days after the implant and declined thereafter. On the other hand, the activity of Pellet C remained high for up to 9 days after the implant. By 11 days after implantation there was no significant difference between the 2 pellets ($p < 0.05$), although animals in both groups were still showing significant abstinence reactions for as long as 15 days after pellet implantation. The activity of Pellet A was the slowest to develop and the fastest to decline. By 12–13 days after implantation the dose of naloxone that was used was insufficient to precipitate even mild withdrawal in these animals.

The severity of the abstinence syndrome as measured by weight loss was similar to the symptom profile (Fig. 2). Analysis of the total area under the curve indicated that weight loss after the injection of naloxone was consistently different in the 3 groups for 15 days following implantation ($p < 0.01$). Animals that were implanted with Pellet C showed a greater weight loss than those implanted with either Pellet A or B ($p < 0.01$), and animals that were implanted with Pellet B showed a greater weight loss than those implanted with Pellet A ($p < 0.05$). These data suggest that for single pellet implanted animals both measures appear to be highly correlated and possess similar capacity for demonstrating differing degrees of dependence.

When animals were implanted with 2 M pellets (either B_2 or C_2) the abstinence syndrome was more severe, and the differences between the 2 types of pellets used were more remarkable (Fig. 1). Analysis of the total area under the curve indicated that the severity of abstinence was greater in groups C_2 and B_2 than it was in groups C_1 and

B_1 ($p < 0.01$ and $p < 0.05$, respectively). While peak activity of a single pellet (B or C) was obtained within 1 day after implantation, peak activity of 2 pellets was not observed until 3 days after implantation. The magnitude of peak dependence following implantation of 2 pellets was approximately twice as great as the magnitude of peak dependence in the latter 2 groups. Subsequent analysis also indicated that the severity of abstinence in group C_2 was dramatically greater than that in group B_2 ($p < 0.01$). Although peak dependence for both groups occurred at the same time, the duration of peak dependence differed greatly. The activity of Pellet B (group B_2) remained high for 3–5 days after the implant and declined rapidly thereafter. On the other hand, the activity of Pellet C (group C_2) ramained high for up to 11 days after implant. Fifteen days after implantation the group differences disappeared, even though significant abstinence behavior was observed in both groups for up to 17 days after pellet implantation.

A similar relationship was evident for body weight loss (Fig. 2). Analysis of the total area under the curve indicated that body weight loss was more severe in groups C_2 and B_2 than it was in groups C_1 and B_1 ($p < 0.01$), but the magnitude of weight loss in group B_2 was not significantly different ($p > 0.05$) from that observed in group C_1. This latter result would suggest that a single pellet of Type C was as efficacious as 2 pellets of Type B for inducing physical dependence, by virtue of the severity of the abstinence syndrome as measured by precipitated weight loss. Subsequent analysis also indicated that weight loss was greater in group C_2 than it was in group B_2 ($p < 0.01$) up until 15 days after implantation. Weight loss in group B_2 remained high for 1–9 days after implantation and declined rapidly thereafter. On the other hand, weight loss in group C_2 remained high for up to 13 days after implantation. By 15 days after implantation the group differences disappeared. By 19 days there was no significant naloxone-precipitated weight loss in either group.

DISCUSSION

The data presented here demonstrate that a large difference in activity may occur between various types of M pellets (50 or 75 mg M) which possess differing specifications such as surface area and pellet hardness. By utilizing 2 separate indices of naloxone-induced abstinence (a symptom profile and body weight loss) we have demonstrated that physical dependence could be detected for as long as 15–17 days after implantation when the most efficacious M pellet was used. In addition, we have demonstrated that it is possible to increase the magnitude as well as prolong the duration of physical dependence by implanting 2 rather than 1 M pellet(s). When 2 M pellets (of the most efficacious type) were implanted, a stable, high degree of physical dependence could be acheived for as long as 11–13 days after implantation, after which body weight loss and symptom profile measures show a rapid decline in their capacity to quantify physical dependence via these measurements.

Even though our experiment was not a parametric analysis of surface area and hardness, our data suggest that these 2 physical attributes may be important characteristics for determining differences in pellet activity. Increased surface area and decreased pellet hardness were associated with greater activity. In general, pellets with larger surface areas and lower hardness rating proved to be the most efficacious. Pellets which possessed low hardness rating were more capable of inducing initial high degrees of physical dependence. At the same time, pellets which possessed large surface areas were capable of maintaining a high degree of stable dependence for longer periods. In this regard, a pellet of large surface area and low hardness rating (Pellet C) had a more consistent activity and a longer duration of action; while a pellet of small surface area and high hardness rating (Pellet A) showed the least activity and the shortest duration of action. As a result of the interplay of these 2 physical parameters, the relative difference between the 3 types of pellet was excentuated during the latter stages of the experiment. These differences were magnified when animals were implanted with 2 pellets.

These data certainly suggest that the use of M pellets of large surface area and low hardness may be useful for inducing higher and more prolonged degrees of physical dependence on opiates that has been previously reported in the literature. Our data indicated that with one type of pellet (C) we could demonstrate stable dependence for as long as 11–13 days after pellet implantation. These results are comparable to the duration of dependence that was reported earlier by Gellert and Sparber [7] using an operant behavior paradigm; but the duration of dependence which we have observed in this study seems to be much longer than that reported by others using similar techniques. Most previous investigators have typically observed that peak dependence subsides after 3–6 days following implantation of a single M pellet [1, 2, 5, 6, 11, 14]. Implantation of 2 or more pellets offers no improvement [3,9]. Since these observations are comparable to that which we observed following implantation of a M pellet of small surface area and high hardness (A), it seems plausible that this type of pellet may have been used by these investigators. Future experiments will be necessary in order to clarify these discrepancies.

In summary, the data which we have presented raise a fundamental issue that is related to investigations of M dependence: First of all, M pellets do vary dramatically in their capacity to induce and maintain physical dependence. Investigators who use the pellet implantation technique should be cognizant of this fact; and secondly, data regarding the specifications of M pellets should be published when reporting the results of experiments dealing with narcotic tolerance and dependence. It is not sufficient to simply describe the standard formula of the tablet.

ACKNOWLEDGEMENT

The authors would like to thank Drs. A. Takemori and M. W. Adler for gifts of two of the morphine pellets (Pellet A and C, respectively) used in these experiments. We would like to thank Endo Laboratories, Inc. (Garden City, NY) for their gift of naloxone HCl.

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