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Potentiation of Morphine Analgesia by Subanesthetic Doses of Pentobarbital

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PONTANI, R. B., N. L. VADLAMANI AND A. L. MISRA. *Potentiation of morphine analgesia by subanesthetic doses of pentobarbital.* PHARMACOL BIOCHEM BEHAV 22(3) 395–398, 1985.—Pentobarbital pretreatment reportedly either inhibits, enhances or has no effect on morphine analgesia. The effect of subanesthetic doses of sodium pentobarbital (8–12 mg kg^{-1}, SC) delivered via a delivery system on analgesia of morphine (5 mg kg^{-1}, SC or 1 mg kg^{-1}, IV) acutely administered 45 min after the sodium pentobarbital pellet implantation was assessed using the warm water (55°C)-induced tail-withdrawal reflex in male Wistar rats. Significant potentiation of morphine analgesia was observed in sodium pentobarbital as compared to the placebo-pelleted animals. Pharmacokinetic or dispositional factors were not involved in this potentiation, which was possibly due to the activation of the descending inhibitory control pathways of nociceptive spinal tail-withdrawal reflex by a combined interaction of two drugs at spinal and supraspinal sites of action, that mediate opiate antinociception.

Pentobarbital-morphine interaction Morphine analgesia Potentiation Rat tail-withdrawal reflex
Sodium pentobarbital delivery system Sodium pentobarbital release [6-^{3}H (n)] Morphine disposition

SUBANESTHETIC doses of pentobarbital are customarily administered before morphine as pre-operative medication in elective surgery. Previous studies have shown that pentobarbital pre-treatment may either inhibit [2, 13, 15], enhance [1, 9, 16] or have no effect [4,5] on analgesia of systemically administered morphine. Light pentobarbital anesthesia has recently been shown [14] to diminish analgesia of morphine administered intracranially but not intrathecally in the rat. In nonhypnotic doses pentobarbital itself has been reported to have negligible or no antinociceptive action [4, 5, 16].

This study was undertaken to determine the effect of subanesthetic doses of pentobarbital released via a delivery system in the rat on the acutely administered morphine analgesia assessed in the nociceptive tail-withdrawal test.

METHOD

Preparation of 10 mg Sodium Pentobarbital Pellets

Cholesterol (360 mg) and glyceryl tristearate (40 mg) were dissolved in chloroform (50 ml) and finely divided sodium pentobarbital powder (100 mg) added to the solution. The suspension was evaporated to dryness *in vacuo* in a Rota Vapor and the residue further dried *in vacuo* overnight. The thoroughly mixed powder (50 mg) was compressed to a cylindrical pellet, diameter 4.5 mm, length 3 mm, surface area 42.6 mm^2 in a Carver Laboratory press (Fred Carver Inc., Summit, NJ). The individual weights of the pellets were within the limits of 95–105% of the average 50 mg weight. Placebo pellets were similarly prepared using cholesterol and glyceryl tristearate.

Estimation of Sodium Pentobarbital Release From Implanted Pellets

Male Wistar rats (150–200 g) were lightly anesthetized with ether and implanted SC with a placebo or a 10 mg sodium pentobarbital pellet in the dorsal area behind the right hind limb. The pellet was pushed away from the incision and the incision sutured. At various times after implantation, the pellets were removed from the animals, thoroughly shaken with ether (10 ml) and then with dilute sodium hydroxide (1 mM, pH 10) in a vibro-mixer. After centrifugation for 10 min, the ether layer was aspirated off and an aliquot of the aqueous phase (1 ml) suitably diluted with water for the measurement of UV absorbance at 240 nm against a reference standard prepared similarly from placebo pellet removed from the animal at the same time as the experimental pellet. The percent doses of drug remaining at the implant site at various times after implantation were calculated.

Nociceptive Testing

The responses of male Wistar rats (150–200 g) to a noxious stimulus were measured using the tail-withdrawal procedure [8] in which the terminal 5 cm of the rat's tail was immersed in a water bath with temperature maintained at 55°C and the time elapsing between immersion and flicking

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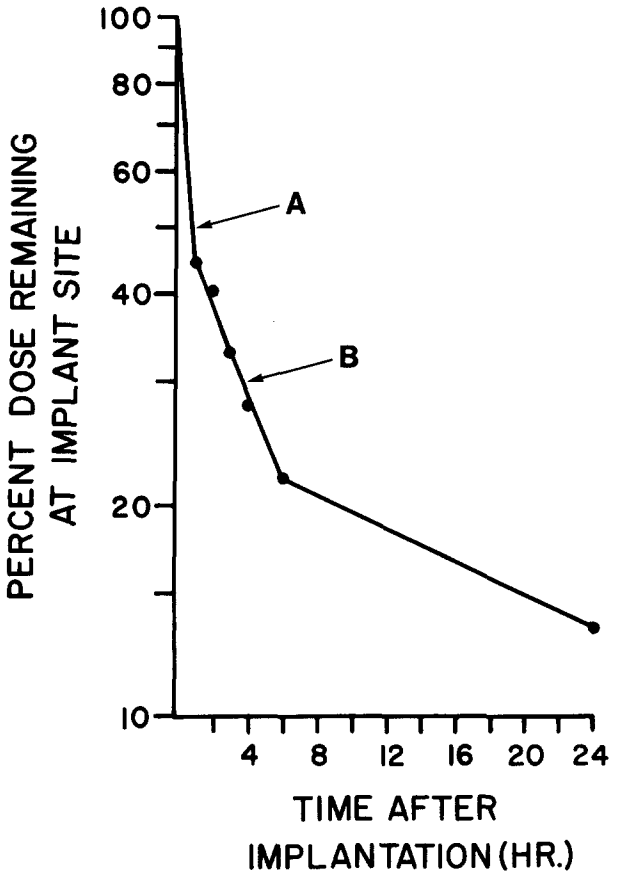

FIG. 1. Semi-logarithmic plot of percent dose remaining in 10 mg sodium pentobarbital pellets at various times after SC implantation in rats. Data represent values from animals implanted with a single pellet at each time (n=1). Morphine injection was given at the time corresponding to point A and the response latencies were measured during the time corresponding to points A and B.

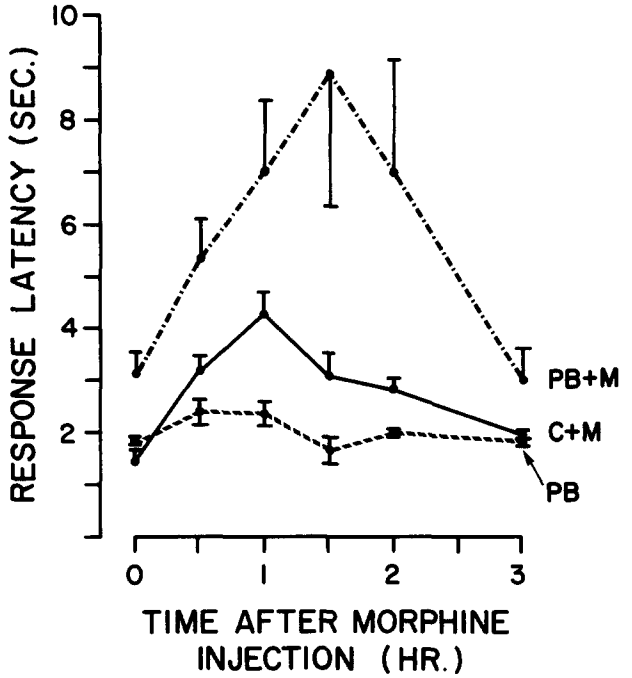

FIG. 2. Tail-withdrawal response latencies (mean±s.e.m., sec) of placebo (C+M) or 10 mg sodium pentobarbital pellet (PB+M) implanted rats (n=5) after a 5 mg kg^{-1} SC injection of morphine; PB represents latencies of sodium pentobarbital pelleted animals without morphine injection. The criteria of reaction of the rats was tail-withdrawal on its immersion in water bath at 55°C and cutoff time was 15 sec. The animals were implanted SC with either a placebo or a 10 mg sodium pentobarbital pellet and 45 min later injected SC with morphine.

of tail was recorded as the reaction time. Average control tail-withdrawal latencies were approximately 1.5–1.8 sec. Threshold response latencies were measured in all animals before placebo or sodium pentobarbital pellet implantation and just before morphine injection. A 5 mg kg^{-1} SC or a 1 mg kg^{-1} IV dose of morphine was administered to two separate groups of placebo and sodium pentobarbital-pelleted rats 45 min after pellet implantation and the response latencies measured at selected times after morphine injection between 10 AM and 3 PM.

Estimation of Morphine Concentrations

Male Wistar rats (150–200 g) were implanted with a placebo or a 10 mg sodium pentobarbital pellet. A 1 mg kg^{-1} IV dose of [6-^{3}H (n)] morphine was injected 45 min after pellet implantation to the two groups of animals (n=5) and 15 min later, the animals were killed to obtain plasma and tissues. Aliquots (2 ml) of plasma (diluted 1:5 with distilled water) or tissue homogenates (10% w/v in 0.5 M HCl) containing 1 ml non-radioactive morphine hydrochloride as carrier (500 μg ml^{-1} as free base) were adjusted to pH 9–9.5 with 1 M NaOH and the solution was buffered with 2 ml 40% w/v K_2 HPO_4 solution and extracted with 15 ml ethylene dichloride containing 30% by volume of n-amyl alcohol as described previously [12]. The *in vitro* recoveries of drug were 95±5% (mean±s.d.) and the identity of the extracted drug was established by t.l.c. of the extracts. Conjugated drug metabolites were not extracted by this procedure. Corrections for quenching of radioactivity in the extracted tissue samples were made using [^{3}H] toluene as internal standard.

Statistical Analyses

The statistical significance of data in the placebo and sodium pentobarbital-pelleted animals was evaluated by Student's *t*-test or the Wilcoxon's ranks sum test.

RESULTS

The SC implantable 10 mg sodium pentobarbital pellets did not produce the loss of righting reflex in rats at any time during implantation. The percent doses of drug remaining at implant site at various times after pellet implantation appear in Fig. 1. The release of drug corresponded to $t_{\frac{1}{2}}$ of 0.8, 5.0, 25.2 hr respectively for the three phases. The points A, B on the curve correspond to the time points during which the response latencies of rats were measured after morphine injection. The release of drug between points A and B corresponded to about 8 to 12 mg kg^{-1} SC dose in rats.

Tail-Withdrawal Response Latencies

Warm water-induced tail withdrawal response latencies in

TABLE 1

COMPARATIVE CONCENTRATIONS* OF MORPHINE IN PLASMA AND SELECTED TISSUES OF PLACEBO OR 10 MG SODIUM PENTOBARBITAL-PELLET IMPLANTED RATS AFTER A 1 mg kg^{-1} DOSE OF [6-^{3}H (n)] MORPHINE BY INTRAVENOUS BOLUS INJECTION

Biofluid or tissue	Morphine concentration	
	Placebo-pelleted animals	Sodium pentobarbital-pelleted animals
Plasma	157 ± 9	130 ± 12
Brain	46 ± 6	47 ± 3
Liver	820 ± 93	855 ± 94
Lung	831 ± 69	941 ± 54
Heart	405 ± 27	388 ± 33

*Data represent mean ± s.e.m. (ng g^{-1} tissue or ml fluid) from 5 animals in each group. The animals were implanted each with either a placebo or a 10 mg sodium pentobarbital pellet and 45 min later injected IV with a 1 mg kg^{-1} dose of [6-^{3}H (n)] morphine. The animals were killed 15 min after injection and plasma and tissues obtained for analyses.

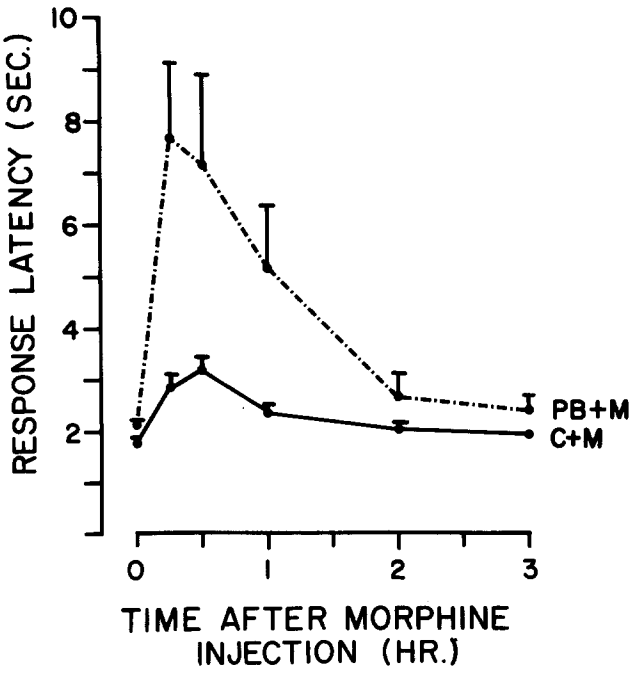

FIG. 3. Tail-withdrawal response latencies (mean±s.e.m., sec) of placebo (C+M) or sodium pentobarbital pellet (PB+M) implanted rats (n=10) after a 1 mg kg^{-1} IV injection of morphine. Other details as in Fig. 2 legend.

animals implanted with placebo and 10 mg sodium pentobarbital pellets after a 5 mg kg^{-1} SC injection of morphine appear in Fig. 2. Pentobarbital-pelleted animals without morphine injection showed a small but significant amount of analgesia 0.5 and 1 hr after implantation, and the response latencies at these times were significantly ($p<0.05$) higher than before pellet implantation. The area under the analgesia-time curve of pentobarbital-pelleted rats after morphine injection (18.17±3.77 sec-hr) was significantly higher ($p<0.01$) than that in placebo-pelleted rats (8.59±0.58 sec-hr). Tail-withdrawal response latencies in animals implanted with placebo and 10 mg sodium pentobarbital pellets after a 1 mg kg^{-1} IV injection of morphine appear in Fig. 3. The area under the analgesia-time curve of pentobarbital-pelleted rats (12.62±2.24 sec-hr) was significantly higher ($p<0.01$) than that in placebo-pelleted animals (6.90±0.25 sec-hr).

Comparative Concentrations of Morphine in Plasma and Tissues of Placebo and Pentobarbital-Pelleted Rats

There were no significant differences in morphine concentrations in brain, plasma and other tissues in the two groups 15 min after IV injection of a 1 mg kg^{-1} dose of [6-^{3}H (n)] morphine (Table 1).

DISCUSSION

This study shows a significant potentiation of morphine analgesia in sodium pentobarbital-pelleted animals as compared to the placebo group. This observation is in accordance with some previous studies [1, 9, 16]. Other studies, however, have either shown inhibition [2, 13, 15] or no effect [4,5] of pentobarbital on morphine analgesia. Subanesthetic doses of pentobarbital itself delivered via our delivery system produced only a small degree of analgesia in tail-withdrawal test during 1 hr after pellet implantation. Negligible or no antinociceptive action has been reported for non-hypnotic doses of pentobarbital in earlier studies [4, 5, 16]. The inconsistencies in the pentobarbital-morphine interaction with special reference to opiate antinociception reported in the literature could be due to the species differences, doses and routes of drug administration and methodological differences in the nature of nociceptive stimulus used and the method of its measurement. Both hypnotic and non-hypnotic doses of different barbiturates and various types of nociceptive stimulus, e.g., pain threshold to tibial pressure in man, mouse tail clip or electric shock or rat-tail flick assays have been used in these earlier studies.

Because of the high aqueous solubility of sodium pentobarbital, the release of drug from the external surface of the lipophilic matrix is quite fast in the initial 40 min and during the period of nociceptive testing and potentiation of morphine analgesia only subanesthetic doses (8–12 mg kg^{-1}, SC) of sodium pentobarbital were released by the delivery system. The amounts of sodium pentobarbital released during 4–6 and 6–24 hr after pellet implantation corresponded to

3–4 and 4–5 mg kg^{-1}, SC doses respectively. At no time during the implantation of these pellets, did the animals lose their righting reflex. In an earlier work [4] a wide range of subanesthetic doses of sodium pentobarbital (4, 8, 16 mg kg^{-1}, SC) in Sprague Dawley rats did not influence analgesia as measured by the tail-compression test of morphine (2–8 mg kg^{-1}, SC) acutely administered 5 min after pentobarbital. This discrepancy in results could possibly be due to procedural differences involving the different nociceptive stimulus used and the mode of administration of sodium pentobarbital. Our sodium pentobarbital pellets and their method of preparation differ from that reported earlier [7] for the rapid induction of functional and dispositional tolerance to pentobarbital in mice, in which all the pentobarbital-pelleted animals lost their righting reflex.

Lack of significant differences in morphine concentrations in brain, plasma and other tissues in placebo and pentobarbital-pelleted rats implied that the potentiation of morphine analgesia by pentobarbital was not due to changes in pharmacokinetic or dispositional factors. Potentiated morphine (5 mg kg^{-1}, SC) analgesia in sodium pentobarbital pelleted animals was abolished completely by naloxone (5 mg kg^{-1}, SC) administered 1 hr after morphine injection. Although the mechanism of this potentiation is not clear at this time, it is conceivable that subanesthetic doses of pentobarbital in conjunction with morphine activate the descending inhibitory control pathways of nociceptive spinal tail-withdrawal reflex due to a combined interaction at the spinal and supraspinal sites of action. It is now well established that such sites mediate opiate antinociception [11, 18, 19]. The activity of these descending inhibitory pathways has also been shown [3] to be susceptible to low doses of pentobarbital. The synergism of the depressant effects of pentobarbital by morphine has been well documented [1, 6, 10, 17], but its role in the potentiation of morphine analgesia as observed in this study remains unclear at this time.

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