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Potentiation of Morphine Analgesia After Pretreatment with Probenecid or Sulfinpyrazone

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MORIN, R. A. AND W. H. LYNESS. *Potentiation of morphine analgesia after pretreatment with probenecid or sulfinpyrazone*. PHARMACOL BIOCHEM BEHAV 18(6) 885–889, 1983.—Pretreatment with uricosuric agents probenecid or sulfinpyrazone potentiate the analgesic effects of morphine sulfate as ascertained using the phenylquinone (PQ)-induced writhing test. Doses of either uricosuric agent at 50 mg/kg had no effect on the number of PQ-induced writhes in test animals while potentiating the analgesic effects of morphine. High doses of probenecid or sulfinpyrazone alone did produce decreases in PQ-induced writhing. Probenecid (50 mg/kg) did not alter hot water tail flick latency nor did it influence morphine analgesia. Attempts to uncover the underlying mechanisms in the uricosuric agent plus morphine attenuation of PQ-induced writhing were directed towards a possible displacement of morphine from plasma binding sites. However, administration of N-methyl-H^3-morphine and estimation of plasma and brain morphine concentrations indicate no differences in the uricosuric drug pretreated groups compared to controls. The conflicting results in the PQ writhing test vs. hot water tail flick might indicate a false positive response in the former test. On the other hand this might be indicative of differing analgesic mechanisms for different types of pain. If the latter is true, this drug interaction may prove clinically useful.

Morphine Phenylquinone writhing Sulfinpyrazone Probenecid

PROBENECID and sulfinpyrazone (Anturane®), both uricosuric agents, have been assumed to have few major actions other than competitive blockade of acid transport processes, predominantly in the kidney. Probenecid also blocks the egress of the acid metabolites of the monoamine neurotransmitters through the choroid plexus and thus has become an invaluable tool in the assessment of cerebral monoamine turnover in both animals and humans (cf., review, [11]). However, it is being recognized that probenecid has other unexplained actions. It has been shown to produce decrements in the locomotor activity of rodents [13], produce hypnosis in birds [1] and produce behavioral changes and decreases in REM (rapid eye movement) sleep time in humans given large doses [12]. Furthermore, probenecid has been shown to reduce the latency to onset and increase the duration of hypnosis to a wide variety of agents in rodents, e.g., ketamine and thiopental [26], phenobarbital and hexobarbital [14] and pentobarbital as well as inhalation halothane [17]. In humans probenecid pretreatment appeared to potentiate the analgesia induced by thiopental [10].

To complicate matters further, probenecid, as several reports suggest alters the synthesis of monoamines in the central nervous system, e.g., 5-hydroxytryptamine (5-HT) by increasing brain tryptophan [24] and dopamine (DA; [2,20]) by an unknown mechanism.

Sulfinpyrazone, likewise, has properties other than blockade of acid transport processes in the kidney tubules. It too potentiates both systemic pentobarbital and inhalation halothane hypnosis [17]. Reports of marked decreases in sudden death after myocardial infarct in patients receiving sulfinpyrazone have prompted renewed interest in this agent.

The purpose of our study was to examine whether pretreatment with probenecid or sulfinpyrazone would potentiate the analgesic effects of morphine in the rat and, if so, to uncover the underlying mechanisms. While we have fallen short of the latter goal, the observed potentiation of morphine analgesia may have immediate clinical usefulness.

METHOD

Animals

Male Charles River Sprague-Dawley rats (Wilmington, MA, 200–250 g) were used throughout these studies. Probenecid (Sigma) and sulfinpyrazone (Ciba-Geigy) used in all the studies were dissolved in dilute sodium hydroxide and pH adjusted to 7.8 for injection. Vehicle consisted of 0.9%

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saline pH 7.8. Morphine sulfate (Sigma) was dissolved in 0.9% saline. Doses are expressed as the salt.

PQ Writhing Test

Phenyl-p-quinone (Sigma) was dissolved in ethanol then diluted with water to make a 0.02% solution (5% ethanol). Volume of drug injected was 1 ml/100 g body weight. The number of writhes was ascertained in the interval of 10–20 min after PQ injection. A description of writhing is described in detail elsewhere [6].

The drugs probenecid, sulfinpyrazone or saline were administered 30 min before PQ injection, i.e., 40 min before commencement of writhing observations. In the morphine potentiation studies, the opiate was administered SC 30 min prior to PQ injection, i.e., 10 min after vehicle, probenecid or sulfinpyrazone. Animals were immediately sacrificed after observation.

Hot Water Tail Flick Test

The other method of testing analgesia was the hot water tail flick [8]. Water temperature was maintained at 52°C. Animals were acclimatized for 1 hour per day on 3 days prior to testing in a restraint device constructed of 3 in. i.d. PVC pipe (length 8 in.). The anterior end of the device was secured with Plexiglas and the posterior end adapted such that fiberglass rods could be lowered to prevent the animal from backing out of the apparatus. All rats had free access to water during acclimatization and testing. The device also had 0.75 in. holes drilled around its circumference at 1.5 in. intervals to insure ventilation. Animals were tested using the distal 5 cm of the tail at 15 min intervals. No change in tail flick response was observed in drug naive rats for periods up to 75 min using this protocol. If a drug naive rat did not remove his tail from the water in 7 sec (0.12 min) the experiment was terminated. In morphine injected rats the experiment was terminated in 20 sec if the rat did not respond.

Determination of Brain and Plasma Morphine

N-methyl-^{3}H-morphine (New England Nuclear, Boston MA, 75 Ci/mmol) was diluted with unlabelled morphine sulfate in saline for SC injection. Rats were pretreated with saline, probenecid or sulfinpyrazone at time zero, administered 1 mg/kg ^{3}H-morphine (0.03 mCi/animal) at 10 min and sacrificed by decapitation at 45 min, a time chosen to represent the midpoint of the PQ-writhing observation period.

The brain was rapidly removed rinsed with cold saline, blotted and weighed. Tissues were then minced and dissolved in Protosol® (New England Nuclear; 6 ml/g tissue). Upon dissolution aliquots of the brain tissue were added to 15 ml ACS scintillation fluid (Amersham, Arlington Heights, IL) and counted in a Tracor Analytic Delta 300 scintillation counter. Trunk blood was collected in ice cold centrifuge tubes at the time of decapitation. Samples were centrifuged at 1000 × g for 20 min (4°C) in a Beckman J2-21 centrifuge. Aliquots of 1.5 ml plasma were added to ACS scintillation fluid and examined as above. Recovery of ^{3}H-morphine was ca. 0.23%/ml plasma and 0.05%/g brain of the total injected dose.

RESULTS

Probenecid Potentiation of Morphine Analgesia

Figure 1 examines altering the dosages of morphine sulfate alone or in combination with probenecid on the number of writhes induced by PQ injection. On the left hand side of the figure a morphine dose reponse was performed. All morphine doses were significantly different from vehicle in reducing the number of PQ-induced writhes (ANOVA, Duncan's Multiple Range test, alpha<0.05). On the bottom left of Fig. 1, animals were pretreated with 50 mg/kg IP probenecid and administered morphine and PQ as before. The number of writhes now observed, compared to the appropriate morphine dose alone, were significantly reduced (Student's t-test, $p<0.05$).

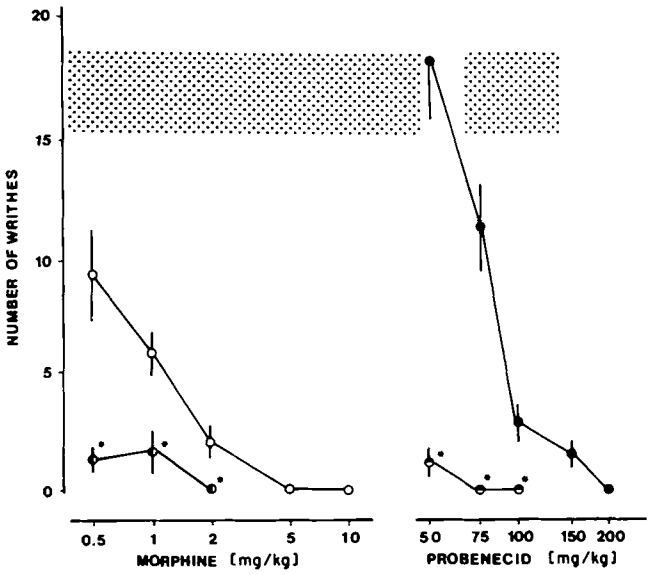

FIG. 1. Potentiation of morphine analgesia by probenecid. The left side of the figure shows varying doses of SC morphine with probenecid (◐, 50 mg/kg IP 10 min prior to morphine) and without probenecid (○, saline injection. The right side of the figures shows that dose related effects of probenecid alone (●) on PQ induced writhing, while the potentiation of morphine-induced analgesia (1 mg/kg SC) 30 min prior to testing (◓) is also shown. Values represent the mean ± 1 SE of 6–10 animals. *Indicates $p<0.05$ versus appropriate control animals in each dose group. Stippled area represents the mean ± 1 SE number of writhes in animals receiving neither probenecid nor morphine sulfate.

On the right-hand side of Fig. 1 the effects of probenecid alone were recorded. It can be seen that a dose efficacious in potentiating morphine analgesia, i.e., 50 mg/kg probenecid, did not produce apparent analgesia alone and only at doses of greater than 100 mg/kg did significant reductions in writhing occur.

In the second part of this figure, a dose of 1.0 mg/kg SC morphine was administered after varying doses of probenecid. All three doses of probenecid in combination with morphine produced significant ($p<0.05$) reductions in PQ-induced writhing compared to the corresponding probenecid dose alone.

Similar experiments were performed using sulfinpyrazone pretreatment. Dose response data using sulfinpyrazone indicated a similarity to probenecid in reducing the number of PQ-induced writhes, i.e., doses greater than 100 mg/kg produced significant reductions ($p<0.05$, data not shown). Figure 2 examines pretreatment with a dose of sulfinpyrazone which was ineffective in reducing PQ-induced writhing, i.e., 50 mg/kg. The time course of drug injections were

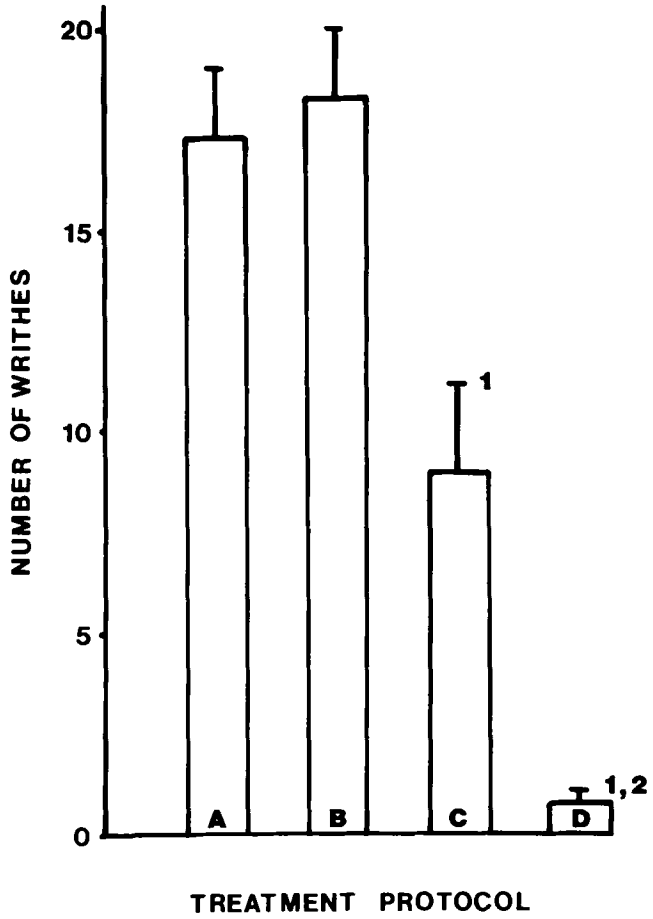

FIG. 2. Potentiation of morphine analgesia after sulfinpyrazone pretreatment. Values represent the mean±SE of 6–9 animals per group identified as follows: (A) drug naive; (B) sulfinpyrazone (50 mg/kg); (C) morphine (1 mg/kg); and (D) sulfinpyrazone (50 mg/kg) and morphine (1 mg/kg). Numbers represent statistical significance (Student's t-test, $p<0.05$) from no drug control (1) and sulfinpyrazone pretreated (2).

TABLE 1

INFLUENCE OF PROBENECID PRETREATMENT ON HOT WATER TAIL FLICK LATENCY WITH AND WITHOUT MORPHINE

Pretreatment			Tail Flick Latency
Probenecid	Morphine	N	(sec)
Vehicle	Vehicle	(5)	4.4 ± 0.3
50 mg/kg	Vehicle	(5)	4.5 ± 0.3
200 mg/kg	Vehicle	(6)	6.6 ± 0.3*
Vehicle	2.0 mg/kg	(7)	16.2 ± 1.1*
50 mg/kg	2.0 mg/kg	(7)	15.4 ± 0.8*

Values shown represent the mean ± 1 S.E. of determinations made 45 min after probenecid or vehicle injection (35 min after morphine or its vehicle injection).

*Indicates $p<0.05$ compared to vehicle-vehicle injection (Student's t-test).

TABLE 2

EFFECT OF URICOSURIC AGENT PRETREATMENT ON BRAIN AND PLASMA MORPHINE CONCENTRATIONS

	Morphine Concentrations		
Pretreatment	Plasma (ng/ml)	Brain (ng/ml)	N
Saline	476 ± 21	70 ± 4	9
Probenecid (50 mg/kg)	486 ± 25	71 ± 6	6
Probenecid (200 mg/kg)	456 ± 20	71 ± 4	6
Sulfinpyrazone (50 mg/kg)	486 ± 43	75 ± 3	5
Sulfinpyrazone (200 mg/kg)	504 ± 31	68 ± 5	7

Rats were pretreated with saline vehicle, probenecid or sulfinpyrazone (IP) and 10 minutes later injected SC with 1.0 mg/kg N-methyl-H^3-morphine. Thirty-five minutes after morphine injection, rats were sacrificed and morphine concentrations determined as described in the Method section.

the same as in Fig. 1. While it can be seen that morphine significantly reduced the number of PQ-induced writhes, the combination of sulfinpyrazone with morphine produced significantly less writhing than morphine alone (Student's t-test, $p<0.05$). The administration of naloxone (5 mg/kg IP) completely antagonized the effects of morphine and sulfinpyrazone, i.e., the number of writhes did not differ from vehicle controls. Even large doses of naloxone (10 mg/kg) however do not antagonize the apparent analgesia produced by large doses of probenecid or sulfinpyrazone (200 mg/kg; data not shown).

A further analgesic test employed was the hot water tail flick latency. Only probenecid was evaluated. Table 1 examines the results. Probenecid alone in doses of 50 mg/kg did not alter tail flick latency. Although the responses of 15 min intervals up to 75 min were recorded only the 45 min test period is shown for simplicity. Administration of 200 mg/kg, a conventionally employed dosage, significantly increased tail flick latency by 150%. This increase in latency was not nearly as great as that of morphine alone, i.e., increase over vehicle controls 350%. The combination of probenecid (50 mg/kg) and morphine did not produce further increases in tail flick latency. Similar studies using 1 mg/kg morphine and probenecid also failed to indicate a potentiation of analgesia (data not shown).

Brain and Plasma Morphine Concentrations After Uricosuric Agent Administration

Either uricosuric drug might prevent narcotic binding or displace morphine from plasma binding sites, the net result might be higher plasma concentrations of free morphine, hence increased brain morphine levels. This simplistic approach to explain the potentiation of narcotic analgesia (PQ writhing test) was refuted by the data of Table 2. Neither brain nor plasma levels of N-methyl-^{3}H-morphine were altered by 50 or 200 mg/kg doses of probenecid or sulfinpyrazone.

DISCUSSION

Doses of probenecid which did not effect the number or characteristics of PQ-induced writhing, when combined with morphine, produced significantly greater reduction in writhing than morphine alone. On the other hand, the combina-

tion of probenecid and morphine was no more effective in reducing hot water tail flick latency than the opiate alone.

These conflicting results could be interpreted two ways. First, it is possible that the decreased writhing observed with the probenecid and morphine combination is the result of a false positive interpretation. Nearly all experimental tests for analgesia have extrapolation problems. The PQ-induced writhing and hot water tail flicks tests are not exceptions (cf., review [6]). A number of compounds with little or no analgesic activity per se reduce PQ writhing (false positives), e.g., antihistamines and LSD [7]. Indeed with higher doses of probenecid alone (>100 mg/kg), significant naloxone insensitive reductions in writhing are observed. It is important to point out here that doses of 100 mg/kg or greater of probenecid produce profound decreases in locomotor activity [13] and food reinforced operant responding (FR-40, Morin and Lyness, unpublished observations). Sulfinpyrazone pretreatment yields comparable results. However, other researchers have found the PQ writhing test to be a reliable index for analgesic potency in man [16].

A second interpretation might be that our results reflect differing mechanisms of analgesia for different types of pain, a concept proposed earlier [4]. Probenecid and sulfinpyrazone through some as yet undefined mechanism might influence only one analgesic mechanism, thus yielding the apparently conflicting results.

Prior to evaluating the hot water tail flick tests, an attempt at defining the mechanism of action of the probenecid-morphine attenuation of PQ writhing was performed. It is known that probenecid and sulfinpyrazone are substantially (>90%) plasma albumin bound and may displace or prevent binding of other pharmacologic agents, in this case morphine. However, even large doses of either uricosuric agent (200 mg/kg) failed to alter brain or plasma ^{3}H-morphine concentrations. Thus a displacement of plasma albumin bound morphine leading to increased brain morphine was eliminated as causative in the potentiation of morphine analgesia.

Other known effects of probenecid, as alluded to earlier, might yield clues as to the apparent mechanism(s) of action. While little is known of sulfinpyrazone, probenecid has been demonstrated to increase brain 5-HT and DA synthesis in the rodent [2,24]. Both monoamines are of import in pain perception and response.

The involvement of 5-HT containing neurons is well documented. In general, depletion of 5-HT (p-chlorophenylalanine, [22]), destruction of 5-HT neurons [19,25] or the use of 5-HT antagonists [26], antagonize the analgesic effects of morphine. Conversely, procedures which elevate brain 5-HT (5-HTP pretreatment, [5]) or intraventricular 5-HT injection potentiate morphine analgesia [21].

Brain dopamine also appears to participate in the mammalian pain response. Depletion of DA nerve terminals has been reported to reduce narcotic analgesia [15], while intraventricularly administered DA potentiates it [3]. Similarly, intra-amygdaloid DA injection attenuates morphine analgesia [18]. On the other hand pretreatment with DA agonists apomorphine or amantadine antagonized while DA antagonists potentiated the analgesic effects of morphine [23]. Apomorphine also decreases tail flick latency in spinal rats [9]. The role of DA in morphine action is thus presently unclear. Possibly, the choice of analgesic tests in these studies was a factor in determining DA action in pain response, again, underscoring the hypothesis of differing neuronal mechanisms involved with different types of pain [4].

It is tempting to speculate that the ability of probenecid to alter the synthesis of both 5-HT and DA might be a causative factor in the apparent potentiation of analgesia produced by the combination of this uricosuric agent with morphine in PQ writhing tests. This hypothesis must, however, await further study.

In conclusion, if the uricosuric agents potentiate analgesic drug effects in higher species, as they do in rodents, a valuable therapeutic drug interaction may be at hand. Clinical evidence suggests this may be the case, e.g., probenecid pretreatment potentiated thiopental anesthesia in patients undergoing a minor surgical procedure [10]. Our studies demonstrate that 0.5 mg/kg morphine with probenecid was as efficacious in reducing PQ-induced writhing as 2.0 mg/kg morphine alone. If these studies can be extrapolated to humans, it might be possible to administer lower doses of morphine, potentially reducing the opiate side effects encountered clinically. Similarly, pain relief experienced with lower doses of morphine might prove useful in combating the tolerance and addiction liability of the opiates, both serious clinical problems in chronic pain relief. However, further research is needed to deny or substantiate these speculations.

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