Analgesia Following Sulfapyridine Administration in Morphine-Pretreated Mice: A Morphine "Reservoir"?[1] (37679)

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(Introduced by A. G. White)

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Two apparently unrelated observations led to the investigations reported here: (1) Sulfapyridine, which is not an analgesic or narcotic agent, potentiates the toxic effects of morphine, codeine, and methadone (1, 2); and (2) Morphine binds to plasma proteins (3–7).

In view of these findings, we postulated that part of the administered morphine binds to blood and/or tissue components, forming pharmacologically inactive complexes, and that sulfapyridine potentiates the toxic effects of morphine by preventing and/or reversing the binding, thus making more free morphine available for pharmacologically active sites.

If this hypothesis is correct, sulfapyridine should also potentiate the analgesic effect of morphine. One should also expect that sulfapyridine, injected in morphine-pretreated animals after the analgesic effect of morphine has worn off, would produce analgesia by dissociating morphine from the inactive morphine complexes. Furthermore, it should be anticipated that sulfapyridine, after repeated administrations, loses its ability to produce analgesia due to depletion of the "morphine reservoir." The results of the experiments reported in this communication are consistent with these anticipations.

Methods and Materials. Inbred male C57BL mice weighing 20–27 g were used. They were housed in metal cages in rooms with controlled temperature (71 ± 2°F) and relative humidity (50%) and were fed Wayne Lab-Blox and water *ad libitum.*

The latency of withdrawal response of mice to heat was selected as an index of analgesia. The hot-plate method described by Eddy and Leimbach (8) was used. A jump off the hot plate (maintained at 54.6°) served as the criterion for acute discomfort in measuring the latency of the response to heat. To avoid desensitization to the heat stimulus, the animals were not permitted to remain on the hot plate for more than 30 sec. All mice that did not respond within this interval were given the maximum score of 30 sec. Baseline responses were established for each animal before each experiment by calculating the mean reaction time of at least two exposures made at time zero and at 5, 15, 30, and 60 min. In the majority of the animals, the baseline responses for this 60-min period were fairly constant. After administration of the test substance to an animal, reaction times were obtained at the same intervals as before and plotted so that a time-response curve could be drawn over the corresponding baseline. The area between the baseline and the time-response curve (reaction-time area) was measured and expressed in min-sec. It was assumed that this area reflected the total analgesic effect of the test substance during the 60-min experimental period. Considering the 30-sec exposure limit, it is evident that the reaction-time area for animals having a baseline response of 10 sec can reach a theoretical maximum of (30 − 10) sec × 60 min = 1200 min-sec. It is also evident that the less the analgesic effect of a sub-

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stance, the more the reaction-time area approaches zero.

Potentiation of the analgesic effect of morphine by sulfapyridine. One group of 10 mice received a subcutaneous injection of sulfapyridine (1.6 g/kg) and a second group of 10 mice was subcutaneously injected with an equal volume of 0.9% saline (control). One hour following this treatment, both groups received a subcutaneous injection of morphine (1 mg/kg). The reaction-time area, reflecting the degree of analgesia, was measured for each animal by the method described above, the mean values computed for each group and their difference statistically evaluated.

Production of analgesia by sulfapyridine in morphine-pretreated mice. A total of 29 mice received a subcutaneous injection of morphine (10 mg/kg). Three days later the animals were randomly divided into two groups. The first group of 19 mice received a subcutaneous injection of sulfapyridine (1.6 g/kg), and the second group consisting of 10 mice was injected with an equal volume of 0.9% saline. Three days after this treatment (six days after morphine administration) both groups were subcutaneously injected with sulfapyridine (1.6 g/kg). Reaction-time areas were measured for all animals before and after each injection. Mean values for each group were computed and the differences statistically evaluated.

The substances used were morphine sulfate (Mallinckrodt Chemical Works) dissolved in 0.9% saline to produce concentrations of 0.1 and 1.0 mg/ml, and sulfapyridine (City Chemical Corporation) sonicated in 0.9% saline to produce a suspension of 40 mg/ml.

Results. Potentiation of the analgesic effect of morphine by sulfapyridine. Table I shows that morphine alone produces analgesia with a mean reaction-time area of 281 min-sec. Following sulfapyridine, which in itself produced no analgesia (mean reaction-time area 27 ± 18 min-sec), the same dose of morphine exhibited a significantly greater analgesic effect both in magnitude and in duration, with a mean reaction-time area of 511 min-sec. The analgesic effect in the group treated with morphine alone peaked at 15 min following the injection and had almost completely worn off by the end of the 1-hr period. In the group treated with sulfapyridine–morphine, analgesia reached the highest level at 30 min following morphine administration and remained at this level for the remainder of the experimental period.

TABLE I. Potentiation of the Analgesic Effect of Morphine by Sulfapyridine.

Treatment	Reaction-time area[a] (min-sec)	p
Morphine (1 mg/kg) alone	281 ± 10[b]	<0.001
Morphine (1 mg/kg) after sulfapyridine (1.6 g/kg)	511 ± 39	

[a] Reaction-time area (see text) reflects degree of analgesia.
[b] Mean ± SEM.

Analgesia produced by sulfapyridine in morphine-pretreated mice. Following the 10 mg/kg dose of morphine, the latency of response to heat reached the maximum allowable time (30 sec) in all animals 15 min after the injection, and remained at this maximum level for the rest of the 60-min experimental period. The mean reaction-time area was 1022 min-sec (Table II). Three days later the analgesic effect of morphine had worn off completely in all mice, as was indicated by the fact that responses to heat had returned to baseline levels. At this time, sulfapyridine administered to the animals of the first group produced considerable analgesia with a mean reaction-time area of 709 min-sec. In 5 animals, the latency of response reached the 30-sec limit in 15 min after sulfapyridine administration, and remained at this level for the rest of the experimental period, thus resulting in a time-response curve indistinguishable from that originally produced by 10 mg/kg morphine. In the remaining animals of this group, the time-response curves showed a progressive rise with three mice reaching the 30-sec cut-off time at the end of the 60-min period. A second sulfapyridine injection (1.6 g/kg) administered to the animals of this group three days after the first sulfapyridine dose (six days after morphine administration) produced analgesia of a significantly lesser degree than

TABLE II. Production of Analgesia by Sulfapyridine in Morphine-Pretreated Mice.

Treatment	Reaction-time area[a] (min-sec)	p
Morphine (10 mg/kg)	1022 ± 31[b]	
Sulfapyridine (1.6 g/kg) 1st dose[c]	709 ± 63	
		<0.001
Sulfapyridine (1.6 g/kg) 2nd dose[d]	106 ± 134	
		<0.001
Sulfapyridine (1.6 g/kg) single dose[e]	465 ± 145	

[a] Reaction-time area (see text) reflects degree of analgesia.

[b] Mean ± SEM.

[c] Administered 3 days after the morphine injection.

[d] Administered 6 days after the morphine injection (3 days after the first sulfapyridine dose).

[e] Administered 6 days after the morphine injection (3 days after saline administration).

the first sulfapyridine injection. In the second group 0.9% saline, administered three days after the morphine injection, did not influence responses to heat. A sulfapyridine injection (1.6 g/kg) administered to this group three days later (six days after morphine administration) produced appreciable analgesia which was of a significantly higher degree than that produced in the first group by the same dose of sulfapyridine injected at the same period (six days) after morphine administration. Table II shows the respective mean reaction-time areas and the statistical significance of their differences. The individual time-response curves following the single sulfapyridine injection given to the second group, in most animals reached a peak at 30 min and then declined but did not return to baseline level by the end of the 60-min period.

Discussion. The results obtained in the first series of experiments indicate that sulfapyridine administered prior to morphine potentiates the analgesic effect of the latter. This is in accord with the hypothesis that part of the administered morphine forms pharmacologically inactive complexes with blood and/or tissue components, and that sulfapyridine potentiates the analgesic effect of morphine by preventing or reversing such binding.

The second series of experiments have shown that sulfapyridine, which is not an analgesic drug, produces analgesia when administered three and even six days after a single morphine injection. This observation is also consistent with the hypothesis that sulfapyridine uncouples morphine from inert complexes, thus making it available for pharmacologically active sites. Supporting this concept regarding the sulfapyridine-induced analgesia is the observation that a second sulfapyridine injection produces minimal or no analgesic effect. It is possible that the first injection depletes the "morphine reservoir" so that very little, if any, morphine remains to be displaced by the second sulfapyridine dose. The fact that this depletion is produced by the first sulfapyridine dose, and is not a spontaneous process, is indicated by the observation that when sulfapyridine administered six days after morphine is not preceded by another sulfapyridine dose, it produces analgesia of significantly greater degree.

The binding of morphine to proteins has already been shown *in vitro* and *in vivo* (3, 4, 6, 7). That such binding may constitute pharmacologically inactive complexes is suggested by the observation that binding of morphine to human serum *in vitro* results in a diminution of the analgesic effect of morphine (6). The ability of sulfapyridine to dissociate morphine from its protein complex would not be a unique phenomenon. Sulfonamides have already been reported to displace other drugs bound to proteins (9). Although it is possible that morphine may bind to several blood and/or tissue components, the likelihood that inactive morphine complexes are formed within the brain merits greater consideration. Prevention or reversal of morphine binding to inert sites in the brain, resulting in greater amounts of free analgesic in the vicinity of active receptors, seems a more likely mechanism of the profound sulfapyridine effects observed in the present investigation. The pharmacologically inactive sites to which morphine binds may correspond to what has been referred to as "silent receptors" (10). These mechanisms, although

plausible, are at present only speculative since, for one thing, no direct evidence has been obtained yet demonstrating that sulfapyridine is capable of displacing morphine from inactive complexes. On the basis of the data presented and of the postulated mechanisms, one could still venture certain theoretical implications.

The possibility, for example, that increased capacity of tissues or blood to form pharmacologically inactive complexes with morphine may contribute to the development of tolerance, is an attractive consideration. It is evident, in that case, that drugs capable of preventing or reversing the process of formation of inactive morphine complexes would influence the degree of tolerance. The recently reported production of antibodies that bind morphine and the diminution of morphine effects in mice injected with a morphine immunogen (7), support the possibility that inactivation of morphine by binding to proteins may play a role in the development of tolerance. It is conceivable that the formation of inactive morphine complexes may involve both immune as well as nonimmune mechanisms.

The findings of this investigation and the postulated mechanisms may provide the basis for new theoretical considerations and potential clinical applications regarding morphine analgesia, tolerance, and addiction (11).

Summary. Sulfapyridine potentiates the analgesic effect of morphine. It also produces analgesia when administered to morphine-pretreated mice long after the analgesic effect of morphine has worn off. Morphine possibly binds to blood and/or tissue components, most likely in the brain, forming pharmacologically inactive complexes, and sulfapyridine prevents and/or reverses the binding, thus freeing active morphine.

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