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EFFECT OF RIFAMPICIN ON DEVELOPMENT OF TOLERANCE TO ANALGESIC ACTIONS OF MORPHINE

Willem Hendrik Gispen[1], William A. Krivoy[2], David de Wied[3] and Emery Zimmermann[4].

[1]Division of Molecular Neurobiology, Institute of Molecular Biology, Padualaan 8, Utrecht, The Netherlands,
[2]National Institute on Drug Abuse, Addiction Research Center, Lexington, Ky., U.S.A.,
[3]Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, Utrecht, The Netherlands,
[4]Department of Anatomy and Brain Research Institute, University of California, School of Medicine, Los Angeles, California, U.S.A.

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SUMMARY

Two series of experiments were performed. In the first series the reaction times of mice were determined using the hotplate technique. In the second series of experiments the reactions of rats were determined using the electric footshock technique. In both series of experiments morphine was observed to alter the responses, and chronic morphine administration resulted in tolerance to these actions of morphine. In both series of experiments, tolerance, defined by these same criteria, was inhibited by the concurrent administration of rifampicin.

Inhibitors of mammalian protein synthesis have been found to reduce development of tolerance to analgesic actions of morphine (M) (1,2). Since the inhibitors tested retard synthesis of a large variety of proteins the observation per se had limited value in constructing or rejecting definitive hypotheses to explain any aspect of development of tolerance to M, and in some instances was used to support conflicting hypotheses. This communication is a description of inhibition of development of tolerance to analgesic actions of M by rifampicin (R). R acts against bacterial DNA-dependent RNA polymerase, and therefore blocks protein synthesis at the transcriptional level (3). As a consequence of its high predilection for inhibiting synthesis of bacterial as opposed to mammalian RNA, R has limited pharmacological activity on mammals, has a low toxicity, and does not appear to influence the central or peripheral nervous systems, or the endocrine system (3).

For the purpose of this communication tolerance to morphine is defined in a restricted sense, i.e., as a reduction of morphine-induced alteration of the response either of mice placed on a hot-plate or of rats given electric footshock (EFS), when this reduction is associated with more than one injection of morphine.

Materials and Methods

Two sets of experiments were performed.

1. The first set of experiments made use of male Swiss Webster mice. The degree of analgesia produced by a standard test dose of M (8 mg/kg), or

placebo, was assessed by measuring each mouse's reaction time on a hot-plate (4). Using this technique tolerance is expressed as a decreased reaction time after the test dose of M.

Four groups of mice were used. During the experiment the mice of Group 1 were injected with M and R vehicle (RV), those of Group 2 with M and R, those of Group 3 with saline (S) and R, and those of Group 4 with S and RV.

On day 1, the initial reaction time of each mouse was measured, i.e., the reaction time on the hot-plate in the absence of any drug or placebo. Mice of Group 1 were then given the test dose of M (8 mg/kg), and RV. Thirty minutes later, they were tested on the hot-plate. That same day, the mice of this group received two additional injections of M, 8 mg/kg/injection, and of RV; the following day they were given three injections of M, 20 mg/kg/injection, and of RV; the third day they were given the test dose of M (8 mg/kg), RV, and thirty minutes later were tested for their reaction time on the hot-plate. Mice of Group 2 were treated with M and tested using the same time and dose sequence described for Group 1, but each injection of M was accompanied by an injection of R, 20 mg/kg. The mice of Groups 3 and 4 were treated and tested in the same temporal sequence as Group 1, the experimental variable being the different drug and placebo combinations stated in the preceding paragraph. Thus, Group 1 was included to demonstrate the course of development of tolerance to analgesic properties of M, Group 2 was included to determine the influence of R on development of this tolerance, and Groups 3 and 4 were included to determine the influence of R and placebo on response times of mice on the hot-plate.

After completion of the experiment the initial reaction time for each mouse was deducted from the post-M reaction times on days one and three, and these values, using analysis of variance (5), were used to compute statistical significance of differences between groups and days.

For injection R was suspended in saline using Tween 80 and the hydrochloride salt of M was dissolved in saline. R or its vehicle was injected intraperitoneally, concurrent to the subcutaneous injection of M or its vehicle. The time between diurnal injections of drug/placebo combinations was approximately four hours, and started at 0800 hours.

2. In the second series of experiments the end-point measured was the response of rats to electric footshock (EFS) using the method described by Evans (6), as modified by Gispen et al. (7). Four groups, each containing eight rats, were used. On the day prior to the experiment all rats were individually placed in a plastic chamber, containing a grid floor, for five minutes. On the first day of the experiment rats of Group 1 were injected with M (10 mg/kg) and with RV, rats of Group 2 were injected with M (10 mg/kg) and with R (20 mg/kg), rats of Group 3 were injected with S and with R (20 mg/kg) and rats of Group 4 were injected with S and with RV. Fifteen minutes after being injected each rat was placed, individually, in the plastic chamber, and the grid floor was electrified for twenty-two separate one second periods, separated by intervals of twenty seconds, and in a fixed random sequence of eleven strengths. The current strength used varied from 33 μA to 300 μA between each one second period but was constant during each one second period. Each time the current was turned on the rat was observed, and its' response recorded as: "no response", "flinch", or "jerk-run-jump". The number of stimuli that produced a given response was expressed as a percent of the total number of potential stimuli presented during a test session, i.e., a percent of twenty-two, and provided the values used for statistical analysis. Each rat received the drug regimen appropriate to his group and was tested once a day for four consecutive days. Upon termination of the experiment the results were analyzed using the Student t-test (8) to test for the significance of differences between groups, and an analysis of variance (orthogonal comparisons were used) (5) to test for the significance of differences within groups but between days.

Results

1. Results of the first series of experiments are presented in Table 1. Comparing the actions of M on day 1 to those on day 3, it may be seen that the reaction time on the hot-plate was more than halved ($p < 0.05$) in the group receiving M plus RV (Group 1), indicating development of tolerance to the analgesic action of M.

TABLE I

Response of mice on hot plate

		Saline	Morphine
Day 1	Rifampicin	2.12 ± 0.65*	22.4 ± 0.87
	Vehicle	1.72 ± 0.94	20.7 ± 1.53
Day 3	Rifampicin	1.70 ± 0.75	20.96± 1.36
	Vehicle	0.14 ± 1.50	3.29± 0.96

*Mean ± S.E.M.
The responses to the treatments indicated are differences between post-drug or placebo, and pre-drug or placebo reaction times. n=10 for all groups except that treated with saline plus vehicle (n=9).

On the other hand, there was no evidence of development of tolerance to the analgesic actions of M when it was given with R (Group 2), i.e., when the results obtained on day 1 were compared to those obtained on day 3, $p > 0.05$. The reaction times on the hot-plate did not differ significantly on day 1 when Groups 1 and 2 were compared ($p > 0.05$). There was no difference in the reaction times between or within Groups 3 or 4 when the data obtained on days 1 and 3 were compared ($p > 0.05$).

2. The results of the second series of experiments are presented in Figure 1A and B. Rats given M plus RV (Group 1) manifested an increased "flinch" response (Figure 1A) and a decreased "jerk-run-jump" response (Figure 1B) to EFS compared (by orthogonal contrast) to Groups 3 and 4 ($p < 0.05$ for each of the four comparisons). These differences in response of Group 1 to EFS plus M gradually diminished, such that the responses on day 2 were significantly ($p < 0.05$) different from those on day 1 and the responses on day 3 and 4 were not significantly different from the responses of Groups 3 and 4 ($p > 0.05$), i.e., tolerance appeared to have developed to the actions of M (Group 1). Rats given M plus R (Group 2) responded to EFS on days 1 and 2 in a manner that could not be statistically distinguished from the responses of Group 1 ($p > 0.05$ for both "flinch" and "jerk-run-jump"). Thus, although there were no detectable differences in the responses to M between Groups 1 and 2 on days 1 and 2, within Group 2 the responses were reduced ($p < 0.05$ for both response categories comparing days 1 and 2 of Group 2), indicating development of tolerance by day 2. However, on days 3 and 4 the responses of Group 2 (Figures 1A and B) were significantly different ($p < 0.05$) from the responses of all other groups. When the data obtained from Groups 3 and 4 were analyzed there were no statistically significant differences ($p > 0.05$) within groups or between days.

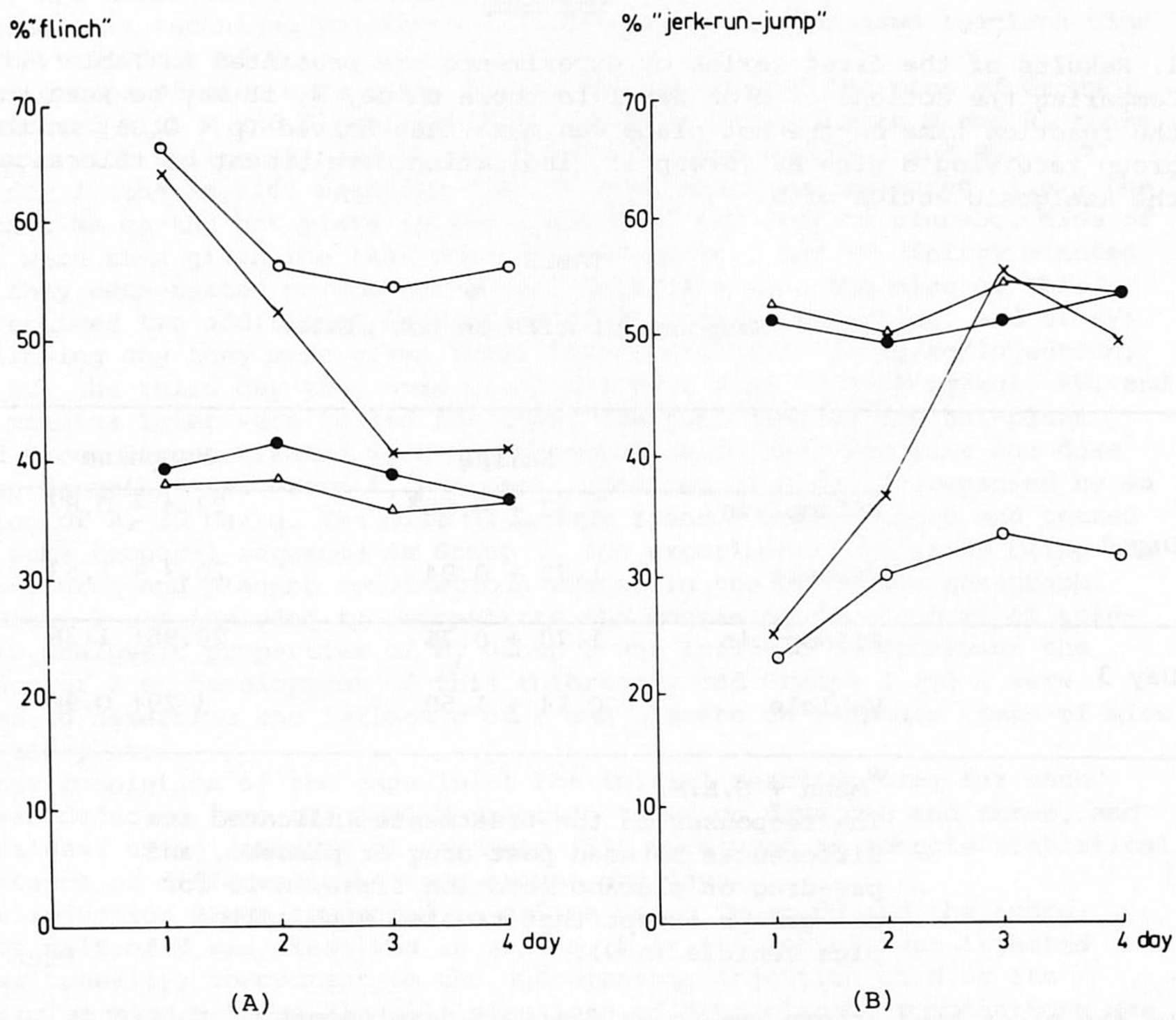

FIG. 1

Response of rats to EFS. Mean percent of stimuli that evoked a "flinch" response (A) and "jerk-run-jump" response (B) after M plus RV (x——x), M plus R (o——o), S plus R (●——●), or S plus RV (Δ——Δ). Standard errors were ± 3.18 or less. N=8 for all groups except M plus R (n=7). For further information see text.

Discussion

Considering the limited use of the concept of tolerance used in this communication, defined in the Introduction, it would appear that using two different species of rodent, and two different techniques, R reduces development of tolerance to M. In both series of experiments Group 1 was included to demonstrate the rate at which tolerance developed to morphine during chronic administration of M and RV. Group 2, in both series of experiments, was included to determine the influence of R on the development of this tolerance. It is obvious that the rate of development of tolerance was at least reduced in the presence of R. This reduction was not likely due to antagonism of M by R since on day 1 the influence of M plus RV was not different from that of M plus R. The apparent influence of R on the development of tolerance to M might also be explained by suggesting that chronic R or RV alters the animals' response to the test procedure. This seems unlikely since neither of the control groups (Groups 3 and 4 of both series of experiments) demonstrated an alteration of response across time. The available data do not allow evaluation of the suggestion that R may be influencing the interaction between the test procedure and M (9). Additional data are also required to determine the

magnitude of the influence of R on the rate of development of tolerance to M since the present experiments were designed only to determine if an interaction exists.

The biochemical, pharmacological and toxicological data available on R (3) indicate its primary action is to inhibit synthesis of bacterial and viral RNA, whereas it has little if any action on mammalian RNA synthesis. If these observations are incomplete and R does in fact inhibit synthesis of some species of mammalian RNA, the same toxicologic and pharmacologic data would suggest the RNA whose synthesis is inhibited by R is either an aberrant species or plays a limited role in normal body function. Therefore, the observations reported herein are in at least partial accord with three current theories intended to explain the development of tolerance to M. First is that proposed by Collier (10) involving the concept of a silent receptor. Second, considering the controversial reports of the influence of R on immune response (11), is that proposed by Cochin (12), suggesting that tolerance to M is due to the production of antibodies. Third, is that suggested by Cohen et al. (1), i.e., that development of tolerance to M could be due to morphine induced production of a species of RNA that is either not generally present in mammals, or is present only in limited quantities.

If R interferes with development of the more general phenomenon of tolerance to M one might reasonably anticipate R would attenuate the M abstinence syndrome. Attempts to investigate this have yielded conflicting results (J. Saelems, personal communication). In one series of experiments using mice, it was found that R antagonized development of physical dependence as demonstrated using a modified mouse jumping test (13). However, in a second series of experiments, it did not.

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