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Naltrexone and the Tail Flick Reflex

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ADVOKAT, C. *Naltrexone and the tail flick reflex.* PHARMAC. BIOCHEM. BEHAV. 15(5) 677–680, 1981.—Rats who received a tail flick test following repeated injections of 5 mg/kg of morphine were significantly more tolerant than rats who were not tested after each injection. Administration of 0.5 mg/kg of naltrexone hydrochloride, prior to the morphine injections, prevented the development of tolerance, increased analgesic latencies and abolished the difference between tested and nontested animals.

Behavioral tolerance Naltrexone Tail flick Opiate analgesia

RECENT results obtained from a variety of response systems, have emphasized the importance of environmental contingencies in the development of tolerance to drugs. These studies have shown that tolerance to several agents, including narcotics [1, 2, 4, 18, 19, 20] alcohol [14] anticholinergics [10, 12, 13, 15] and others [5,9], can be similarly modified by the context associated with chronic drug administration and assessment. Such diversity suggests that contextual modulation of tolerance represents a nonspecific, general adaptation to chronic pharmacological insult. That is, the process(es) responsible for the contextual effects appear to be independent of the specific agent employed to produce tolerance.

One means of independently assessing the contribution of environment and pharmacology in tolerance development would be to block the action of the pharmacological stimulus while maintaining the association between the drug administration context and assessment procedure. The studies presented here were designed to accomplish this goal by using the opiate antagonist naltrexone to block the analgesic action of morphine. Administration of the antagonist should block the pharmacological, narcotic response, thereby preventing opiate tolerance. By comparing the degree of tolerance obtained following naltrexone pretreatment, with that obtained after saline pretreatment, it should be possible to evaluate the influence of contextual cues on behavioral tolerance.

GENERAL METHOD

Subjects

A total of 193 naive, male, albino Sprague-Dawley derived rats (King Laboratories, Oregon, WI) served as experimental animals. The rats weighed 250–300 g at the start of each experiment and were housed four to six to a cage with ad lib access to food and water.

Drugs

Morphine sulfate and naltrexone hydrochloride were dissolved in 0.9% saline and injected either intraperitoneally (IP) or subcutaneously (SC) as noted, in a volume of 0.1 ml per 100 g of body weight; the same volume was used for saline injections.

Analgesic Tests

The tail flick (TF) response was automatically recorded and defined as the elapsed time between onset of a high intensity light beam focused on the tail and the reflex withdrawal (flick) response. Each test consisted of the mean latency of three successive trials. For each trial the tail was replaced on the apparatus so that a different patch of skin was stimulated. To avoid excessive injury a cut off value of 14 sec was automatically imposed on the response.

Statistical Analyses

In the first experiment, all tail flick latency (TFL) measurements were converted to a ratio of the increase in TLF after morphine to the maximum possible increase. This ratio, called the Analgesia Index (AI) was calculated as follows:

$$AI = \frac{\text{TFL after morphine} - \text{control TFL}}{\text{Cut-off time (14 sec)} - \text{control TFL}}$$

As a result of this transformation, the data could be analyzed by a two-way analysis of variance.

In subsequent studies the experimental design required that half of the animals remain untested until the final session. As a result, no baseline control measurements could be obtained from those nontested groups and no Analgesic Index calculated. In addition, many animals in these experiments were maximally analgesic and reached the 14 sec limit imposed on the response. Therefore, only nonparametric statistics were appropriate for these data and the results were analyzed by the Kruskal-Wallis one-way analysis of variance and the Mann-Whitney U-test [17]. All significance levels are two-tailed.

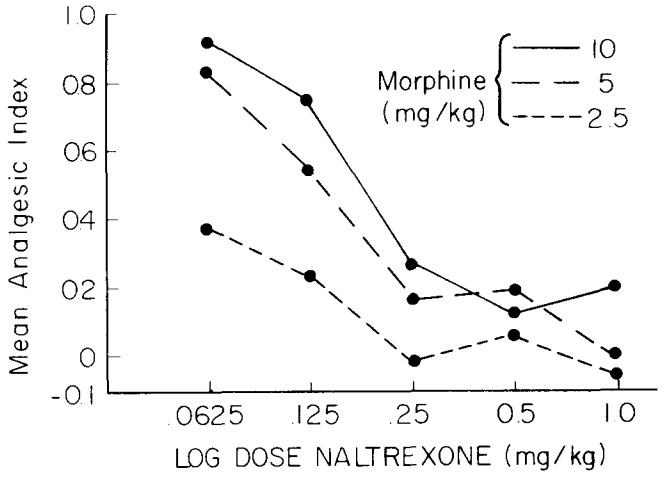

FIG. 1. Mean analgesic Index of rats following a subcutaneous dose of either 10, 5 or 2.5 mg/kg of morphine, as a function of prior interperitoneal administration of one of five doses of naltrexone (N=7 for each point).

EXPERIMENT 1: NALTREXONE ANTAGONISM OF TAIL FLICK

METHOD

In the first experiment it was necessary to determine those doses of naltrexone which would effectively antagonize morphine analgesia on the tail flick test, since this information was not available in the literature [6,16]. All rats received a baseline tail flick test, followed by an IP injection of one of five naltrexone doses, 0.0625, 0.125, 0.25, 0.5, or 1.0 mg/kg. Approximately 20 min after this initial injection, all rats received a second, SC injection, of one of three morphine doses, 2.5, 5.0 or 10.0 mg/kg. Thirty min after the second injection, all rats were given a second tail flick test. Therefore, the first experiment contained 15 groups (N=7 in each case) each of which received one of the 15 drug combinations.

RESULTS AND DISCUSSION

The results of the first experiment, summarizing the dose response relationship of morphine and naltrexone, are shown in Fig. 1. There was no difference in control, pre-drug latencies among the groups in the first experiment, the median baseline latencies of all 15 groups ranged from 2.2 to 3.2 sec. As indicated in the figure, tail flick latencies were a function of both drug doses. Statistical analysis confirmed this observation. There was a signficiant effect of naltrexone, $F=25$, $p<0.001$, and of morphine, $F=14$, $p<0.001$, with no interaction, $F=1.3$, NS. Visual inspection of Fig. 1 suggests that analgesic tail flick latencies are reliably blocked by doses of naltrexone equal to or greater than 0.25 mg/kg. As a result of these data, the dose combination of 5 mg/kg of morphine and 0.5 mg/kg naltrexone was chosen for further study.

EXPERIMENT 2: NALTREXONE AND OPIATE TOLERANCE

METHOD

In the second experiment a total of 33 rats were injected with morphine (5 mg/kg, SC) three times a week, on Monday, Wednesday and Friday, for six sessions. Approximately 20 min prior to each of these injections, all rats received an additional injection of either saline (N=15) or naltrexone (0.5 mg/kg; N=18). Each of these two main conditions consisted of two groups. For one of these groups, the Nontested (NT) condition, all injections of morphine, saline (N=7) and naltrexone (N=9) occurred in the vivarium, where the animals were housed. These rats were not exposed to the laboratory environment or the tail flick at this time. The other group received their morphine plus saline (N=8) or naltrexone (N=9) injection in the laboratory. These rats were tested on the tail flick 30 min after each morphine administration, and constituted the Tested (T) condition.

On the seventh test session, all rats were brought to the laboratory where they received a saline injection prior to morphine administration. Thirty min later, all rats were tested on the tail flick.

RESULTS AND DISCUSSION

The results of this study are shown in the top half of Fig. 2. On the first session, indicated by the dashed lines in the figure, saline pretreated animals were significantly more analgesic than naltrexone pretreated animals ($U=3$, $p<0.002$); by the sixth session there was no difference between these two groups, the saline pretreated animals were behaviorally tolerant. The top half of Fig. 2 also summarizes the results obtained on the seventh session, when naltrexone was replaced with saline. There was a significant difference among the four groups on this test ($H=36$; $p<0.001$). Data shown on the left half of the figure replicates earlier reports demonstrating that morphine tolerance of TF is specific to the environmental context and assessment procedure with which the opiate has been paired [2]. That is, in spite of the fact that all animals received the same amount of morphine, on the same schedule, animals whose injections were associated with the laboratory environment and tail flick test (group T) were tolerant, relative to the nontested group (NT) ($U=1$, $p<0.001$).

As shown on the upper right side of Fig. 2, however, this relationship was not found in animals pretreated with naltrexone. Previous morphine-test pairings did not attenuate the subsequent effects of morphine, if such pairings were preceded by naltrexone administration. Naltrexone appeared to antagonize both, the influence of environmental context, as well as the pharmacological consequences, of chronic opiate administration.

These results suggest that the influence of the administration and assessment context on TF tolerance appears to be mediated through an opiate mechanism, rather than an independent, nonopiate related process. It remains to be seen whether this is the case for other opiate responses and whether contextual modulation of tolerance to other drugs is similarly effected through each respective pharmacological substrate.

The results of Experiment 2 show an additional effect; an unexpectedly profound analgesia in naltrexone pretreated animals. In both the Tested ($U=1$; $p<0.002$) and the Non-

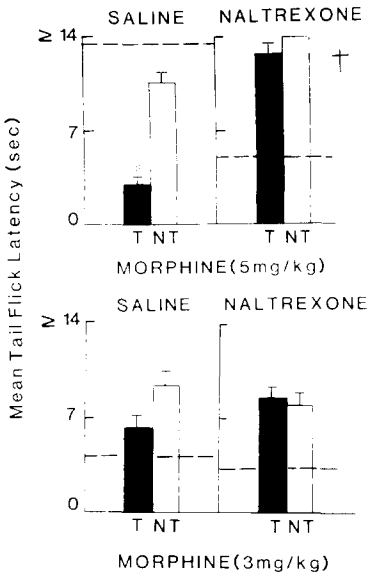

FIG. 2.(Top) Mean tail flick latency (±standard error) of four groups of rats, 30 min after their seventh morphine injection (5 mg/kg). Prior to each of the six previous opiate injections, two of these groups had received a saline injection (SALINE) and two of the groups had received a naltrexone (0.5 mg/kg) injection (NALTREXONE). One of the two SALINE and NALTREXONE groups was tested on the tail flick apparatus after each of the six previous injection regimens (T); the other group was not exposed to or tested on the tail flick apparatus after the previous injections (NT). The dashed lines indicate the latencies of each of the tested groups on the first session. †=significant difference between naltrexone and saline for both Tested ($p<0.002$) and Nontested ($p<0.02$) conditions. *=significant difference between Tested and Nontested SALINE groups ($p<0.001$).

(Bottom) Mean tail flick latency (±standard error) of four groups of rats, 30 min after morphine administration(3 mg/kg). Two groups (SALINE) had received six previous saline injections, two groups (NALTREXONE) had received six previous naltrexone injections (0.5 mg/kg). Each of these two main groups were subdivided; one subgroup received a tail flick test after each injection (T) the other subgroup was not previously exposed to or tested on the tail flick (NT). Dashed lines indicate latencies of the tested groups on the first session.

tested conditions ($U=4.5$, $p<0.02$) naltrexone pretreatment antagonized the decline in tail flick latency evident in the saline pretreated animals. Similar results, demonstrating increases in both opiate and stress induced analgesia, as well as opiate binding sites, following chronic antagonist administration, have previously been reported [3, 11,22]. Those reports have interpreted such facilitation as a purely pharmacological phenomenon. An alternate possiblity existed, however, that the opiate activity of the antagonist itself might have been influenced by the environmental stimuli accompanying drug administration.

For example, chronic naltrexone pretreatment might have engendered a compensatory conditioned response in a manner analogous to that previously demonstrated for morphine [18]. In parallel with the hyperalgesic compensatory response produced by chronic opiate administration, pretreatment with the antagonist might have induced a compensatory analgesic reaction, which was revealed on the last test session when saline replaced naltrexone. Or, perhaps, more simply, the novelty of the saline injection, in conjunction with the assessment context, might have enhanced the analgesic responses of the naltrexone groups on the final session. The next two experiments examined these alternatives by determining the effects of naltrexone pretreatment on both, the acute analgesic action of morphine (Experiment 3) and nociceptive responsivity (Experiment 4).

EXPERIMENT 3: NALTREXONE AND OPIATE ANALGESIA

METHOD

In the third experiment, a total of 35 rats received a SC injection of either saline (N=17) or 0.5 mg/kg of naltrexone (N=18) three times a week, on Monday, Wednesday and Friday, for six sessions. Each of these two main conditions consisted of two groups. One group in the saline (N=9) and naltrexone condition (N=9) was injected in the vivarium and was not exposed to the laboratory environment or the tail flick test; these were the nontested groups (NT). The other group in the saline (N=8) and naltrexone condition (N=9) was brought to the laboratory for the injections, which were followed thirty minutes later by a tail flick test; these were the tested groups (T). On the seventh test session all rats were brought to the laboratory, where they received a SC injection of morphine (3 mg/kg) followed 30 min later by a tail flick test. The dose of morphine was reduced to 3 mg/kg to insure that analgesic responses would be less than maximum.

RESULTS AND DISCUSSION

The results of this experiment are summarized in the bottom half of Fig. 2.

In contrast to the results of the previous study there was no statistical difference among the four groups in this experiment. The acute analgesic effect of a single morphine injection was the same in naltrexone pretreated as in saline pretreated animals. It should be noted, however, (on the lower left half of Fig. 2) that saline pretreated animals, who were tested, were less analgesic than their nontested counterparts, although this difference was not large enough to produce a significant difference among the four groups. Similar effects have been reported with the hot plate test; in some [7,8] but not all [1, 8, 20] cases, pre-exposure to the hot plate attenuated opiate analgesia.

EXPERIMENT 4: NALTREXONE AND NOCICEPTION

METHOD

In the fourth experiment, a total of 20 rats received a SC injection of either saline (N=10) or 1.0 mg/kg naltrexone (N=10) three times a week, on Monday, Wednesday and Friday for ten sessions. All injections occurred in the laboratory and were followed by a tail flick test 30 min later. On the eleventh session all rats received a saline injection prior to the regular tail flick test.

RESULTS AND DISCUSSION

The two groups in this experiment never differed in their tail flick latencies. On session ten, the mean latency of the saline group was 2.4 sec, and of the naltrexone group, 2.5

sec. On session eleven the respective latencies were 2.9 and 3.1 sec. No evidence of analgesia was obtained on the tail flick test from animals chronically pretreated and tested with naltrexone either prior to or after a saline injection.

GENERAL DISCUSSION

In concert, the results of these experiments indicate that both contextual as well as pharmacological processes mediating opiate tolerance may be antagonized by opiate blockade. Furthermore, chronic opiate blockade may actually potentiate subsequent agonist activity. Such potentiation is not a function of prior exposure to either the antagonist alone or the assessment context, but is mediated by those, as yet unknown, processes initiated by the interaction of opiate agonists and antagonists.

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