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RAPID COMMUNICATION

Is Tolerance to Intrathecal Morphine in Intact Rats Supraspinally Mediated?[1]

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ADVOKAT, C. AND J. MAGOUN. *Is tolerance to intrathecal morphine in intact rats supraspinally mediated?* PHARMACOL BIOCHEM BEHAV **39**(3) 761–763, 1991.—The effect of repeated intrathecal (IT) injections of 5 μg of morphine on the tail flick (TF) was determined in rats that were tested either 0.5 h or 5.0 h after administration on each of five successive days. Tolerance developed rapidly in animals tested 5.0 h after each injection. Animals tested 0.5 h after each injection did not become tolerant. Animals that were tested 5.0 h after an intrathecal saline injection on the first four days were also tolerant to a 5 μg dose of morphine on the fifth day. These data are discussed in the context of previous conflicting reports concerning tolerance to intrathecal morphine. It is suggested that, under certain conditions, tolerance to intermittent intrathecal morphine administration may be due to a supraspinal opiate action.

Spinal opiate analgesia Intrathecal morphine Opiate tolerance Tail flick

ALTHOUGH it is well established that morphine can produce analgesia by a direct action at the spinal cord, there is some disagreement concerning the development of tolerance to this effect during chronic spinal administration. Continuous spinal infusion of morphine induces tolerance on the hot plate and tail flick analgesic procedures (3, 11, 18–20). Acute, intermittent IT morphine injections also produce tolerance when supraspinally mediated behaviors, such as the hot plate and shock titration tests, are used to assess analgesia (3, 20, 21). However, there is some disparity regarding the effect of acute IT morphine injections on the spinally mediated TF response.

In the initial report, twice daily IT injections of 15 μg of morphine in rats, administered approximately 12 h apart, produced tolerance within three days (20), and this result was essentially replicated in a recent report under similar experimental conditions (15). Tolerance was also demonstrated in rats injected once daily with 32.0 μg of morphine for seven days and tested on the first and last days (18). On each of these two test days, subjects were injected with IT saline and repeatedly assessed with the TF test prior to morphine administration. Finally, tolerance has also been produced within a single experimental session by cumulative IT injections of increasing doses every 10 min (19).

In contrast to these positive findings, we previously reported that daily IT injections of either 5, 15, or 30 μg of morphine did not induce tolerance on the TF in rats for at least four days (2). While the explanation for these disparate results is not obvious, we have proposed that previous demonstrations of tolerance might be due to a supraspinal action of the opiate. We suggested that, under certain conditions, intrathecally injected morphine might gain access to supraspinal sites and that such exposure promoted the development of tolerance (2). This might be more likely to occur when high doses (15 μg or greater) are administered for sufficiently long periods of time (more than four or five days) or at shorter time periods (less than 24-h intervals). Furthermore, it is well known that behavioral tests in themselves can reduce the subsequent analgesic effect of systemic and supraspinal morphine injections. Behavioral tests alone, however, do not affect the analgesic response to IT morphine, when animals are assessed 30 to 40 min after injection (17), suggesting that the expression of "behavioral" tolerance in previously tested animals is also mediated by a supraspinal action of morphine. Therefore, the present experiments were conducted to determine whether tolerance to a low dose of IT morphine might be preferentially induced to intermittent IT morphine injections by extending the postinjection-test period. It was predicted that, if sufficient time were allowed for morphine to reach the brain before the animals were tested, then tolerance might develop rapidly to repeated IT morphine injections and that "behavioral" tolerance would be observed.

The experiments were performed on Sprague-Dawley derived rats (Holtzman Laboratories, Madison, WI), weighing 300–350 g. All rats were implanted, under ether anesthesia, with intrathecal catheters (PE-10) which terminated at the lumbar enlargement. Any rat exhibiting signs of impairment, i.e., evidence of crippling in any limb either following surgery (N = 10–20%) or after any injection (N = 0), was eliminated from the study. After surgery, rats were housed individually in suspended, stainless

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steel cages in a colony room maintained on a 12:12 light-dark cycle, with dark onset at 1700 h. Food and water were available ad lib.

Antinociception was determined by reactivity to a noxious thermal stimulus provided by an overhead projector bulb applied to the ventral surface of the tail. The TF latency was measured automatically by a photocell placed above the light aperture, with a 14 s limit to prevent tissue damage from repeated trials. Each score consisted of the mean response to three determinations, obtained by successive stimulation of a different patch of skin on each trial. Baseline scores were obtained at the beginning of each experiment, before the first injection, approximately seven days after cannulation. Statistical analyses (analyses of variance, Newman-Keuls test of the difference between means and Student's *t*-test) were performed on the group means with the aid of a computer program (Crunch Interactive Statistical Program). The results are expressed as the mean ± SEM TF latency, in s, and differences among the groups were considered to be significant at $p = 0.05$ or less.

For IT injections, solutions of morphine sulfate (Penick Corp., Lyndhurst, NJ) were made such that the injection volume of 10 μl contained 5 μg, and each injection was followed by a 10-μg wash of saline. When saline alone was administered, the injection volume was 20 μl. Injections were performed manually using a 50-microliter Hamilton syringe over 2–3 min.

The antinociceptive effect of 5 μg of morphine was assessed at either 0.5 h (Group 0.5 h-mor, N = 5) or 5.0 h (Group 5.0 h-mor, N = 12) on day one and N = 7 on days two through five) after administration on each of five successive days. This difference in the number of subjects between day 1 and subsequent days is due to the fact that the first five rats were only tested once, to determine whether analgesia would last for 5 h. It was only after these data were collected that the decision was made to add more subjects, in order to replicate this result, and to examine tolerance. A third group was assessed 5.0 h after saline injections on each of the first four days (Group 5.0 h-sal, N = 8). On the fifth day, this group was tested 5.0 h after an injection of 5 μg of morphine. An additional group was also injected with 5 μg of morphine and tested in one session at 0.5, 1.0, 3.0 and 5.0 h after administration (Group 5.0 h-mor + test, N = 6). All five-hour sessions were conducted between 9:00–11:00 a.m. (injections) and 2–4 p.m. (tests). The 0.5-h sessions were conducted between 12:00 and 2:00 p.m.

There was no difference among the four groups in baseline TF latency prior to their respective injections (mean latency, N = 31, 4.36 ± 0.78). Figure 1 summarizes the results of the first four test sessions. Separate within subject *t*-tests indicated a significant increase in latency over their respective baselines, for each group after the first injection [Group 5.0 h-sal, $t(7) = 6.6 \pm 0.57$, $p = 0.0087$; Group 5.0 h-mor, $t(11) = 10.4 \pm 0.75$, $p = 0.0001$; Group 0.5 h-mor, $t(4) = 10.3 \pm 0.54$, $p = 0.0003$]. However there was also a significant difference among the three groups, $F(2,22) = 8.71$, $p = 0.0016$. The scores of each of the two morphine groups were significantly greater than that of the saline group ($p < 0.01$ in each case) but not from each other. This indicates that the increase in latency produced by the first morphine injection was greater than that produced by saline alone, and that the analgesic response to 5 μg of morphine was the same at the two postinjection intervals.

Figure 1 also shows that the latencies of both groups tested 5.0 h after their respective injections declined significantly over the four daily sessions [Group 5.0 h-sal, $F(3,21) = 3.26$, $p = 0.04$; Group 5.0 h-mor, $F(3,18) = 6.64$, $p = 0.0032$]. In contrast, the scores of animals tested 0.5 h after each injection did not decrease during this time.

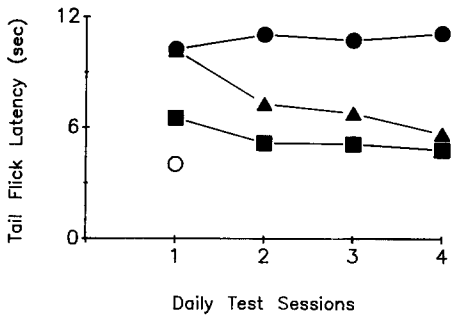

FIG. 1. Mean tail flick latency of three groups of rats on each of four successive daily test sessions. One group was tested 5.0 hours after intrathecal saline injections (filled squares), one group was tested 5.0 hours after intrathecal morphine injections (5 μg, filled triangles) and one group was tested 0.5 hours after intrathecal morphine injections (5 μg, filled circles). The open circle indicates the baseline latency of these three groups (4.1 ± 0.3 s).

Figure 2 summarizes the results of day five, when all three groups were injected with 5 μg of morphine. There was a significant difference among the groups, $F(2,17) = 20.4$, $p < 0.0001$. In this case, the scores of each of the two groups tested 5.0 h after morphine were significantly lower than the mean of those animals tested 0.5 h after morphine administration ($p < 0.01$ in each case). A second analysis of the data from Group 0.5 h-mor, which included the results of the fifth injection, showed no decline across all five sessions, $F(4,16) = 0.537$.

An additional comparison was made to determine whether repeated TF tests, administered during the 5.0-h period after a single morphine injection, would affect the latency obtained at 5.0 h. It was found that the mean latency of Group 5.0 h-mor + test was significantly lower than the latency of Group 5.0 h-mor, at the common test interval of 5.0 h [6.02 ± 0.71 vs. 10.4 ± 0.75, $t(16) = 3.69$, $p = 0.002$].

The results of this study are consistent with a previous report from this laboratory (2) which showed that the antinociceptive effect of repeated acute IT injections of 5 μg of morphine does not decline for at least five days, when animals are tested on the TF approximately 30 min after each injection. The data also support numerous demonstrations of the long-lasting duration of analgesia following a single IT morphine injection. In this study, the same dose of morphine produced a comparable analgesic response at 0.5 and 5.0 h after injection. However, this analgesic

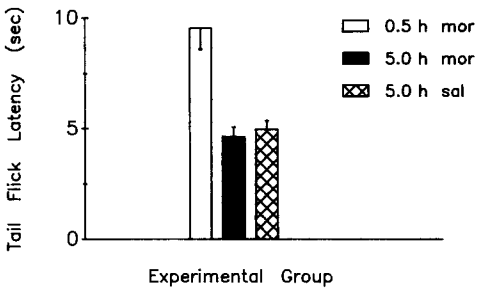

FIG. 2. Mean tail flick latency of the three groups of rats, described in Fig. 1, on the fifth experimental session, tested at their respective postinjection time points, after an intrathecal injection of 5 μg of morphine.

response diverged during subsequent daily assessments. Tolerance developed rapidly when animals were tested 5.0 h after injection, whereas no tolerance was observed when the TF was assessed 30 min after injection.

The fact that tolerance could be induced to IT morphine is also consistent with previous reports, described earlier. However, one interpretation of those data, as well as the present results, is that tolerance was mediated by a supraspinal opiate action. That is, during repeated spinal administration, some portion of the drug may reach the brain, and the reduced effect of subsequent morphine injections when animals are repeatedly injected and tested may be due to such prior exposure.

Against this interpretation, it has been argued, on the basis of results obtained with intrathecal injections of dye or radioactive morphine, that the opiate is restricted to the spinal cord, not only after a single IT injection (20) but also after multiple injections (15). On the other hand, it has also been reported that, within 15 min after intrathecal injection in rats, dye can be detected in the fourth ventricle (12). Furthermore, opiate-induced side effects, which are supraspinally mediated, such as nausea, vomiting and respiratory depression have also been described in several clinical papers after a single IT injection (5, 6, 8–10, 16). Although in many of these cases the doses were high, such results have occurred after injection of as little as 1 mg of morphine (6). The fact that these side effects are delayed for up to four to six hours after injection indicates that they are a result of rostral CSF flow rather than uptake into the systemic circulation (7, 13, 14). Furthermore, we have previously reported that repeated daily IT morphine injections can produce a conditioned place preference as well as locomotor hyperactivity, effects which also indicate a supraspinal action (1).

The development of tolerance to morphine may also be influenced by repeated performance of the nociceptive response. Such "behavioral" tolerance is expressed when morphine is administered either systemically or supraspinally but not when animals are tested 30 to 40 min after an IT injection (17). In the present study, behavioral tolerance was also obtained in response to IT morphine when assessments were made 5.0 h after injection. It is therefore proposed that the differential expression of behavioral tolerance after a 5.0-h as opposed to a 0.5-h postinjection-test interval, is due to a supraspinal action of the drug at the longer time point. This interpretation is also consistent with the fact that, in the present study, repeated TF assessment during a single 5.0-h postinjection interval also produced tolerance, relative to the latencies of animals that were not tested within that time period. This finding implies that animals that have practiced the nociceptive response may exhibit tolerance to IT morphine if they are tested under conditions which foster supraspinal exposure.

It should, perhaps, be emphasized that we are not proposing that the spinal cord does not become tolerant to morphine. It is clear that tolerance develops to both systemic and IT morphine in spinal animals (2,4). However, the apparent conflict in results concerning tolerance to IT morphine in intact animals may be reconciled by considering the possibility that this phenomenon involves a supraspinal process.

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