Pharmacology Biochemistry & Behavior, Vol. 15, pp. 637–640, 1981. Printed in the U.S.A.

Apparent Lack of Tolerance in the Formalin Test Suggests Different Mechanisms for Morphine Analgesia in Different Types of Pain

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Received 10 March 1981

ABBOTT, F. V., K. B. J. FRANKLIN, R. J. LUDWICK AND R. MELZACK. *Apparent lack of tolerance in the Formalin test suggests different mechanisms for morphine analgesia in different types of pain.* PHARMAC. BIOCHEM. BEHAV. 15(4) 637–640, 1981.—Tolerance to morphine analgesia was examined using the Formalin test in which pain lasting about 2 hrs associated with minor tissue injury is produced by subcutaneous injection of dilute Formalin. To distinguish behavioral from pharmacological tolerance, different groups of rats received their daily morphine injection (7 mg/kg) in the test environment or in their home environment for 5 days. Another group of rats was given morphine for 15 days in the home cage followed by 5 days in the test environment. None of the morphine injected groups differed from saline injected control groups in the amount of analgesia. These findings add to previous evidence that the Formalin test measures a type of pain which is different from that assessed in withdrawal reflex tests, and which more closely resembles clinical pain in man. Moreover, the fact that analgesia in the Formalin test shows little tolerance while analgesia in withdrawal tests shows rapid tolerance suggests that the underlying neural mechanisms are different.

Morphine Opiates Narcotics Tolerance Behavioral tolerance Formalin test Tonic pain Pain Analgesia

IT is well known that repeated administration of morphine leads to a progressive decrease in many of its effects. One large and dramatic decrease is due to conditioned or situation-specific tolerance to morphine's analgesic effect. This tolerance develops if the animal is exposed to both the drug and the testing environment 2 to 5 times and leads to the almost complete disappearance of the analgesic effect [2, 3, 22, 28, 29]. However, when narcotic analgesics are administered repeatedly to humans for clinical pain, such as cancer pain, there is rarely a large decrease in the analgesic potency during the first few days of treatment. Instead, the degree of tolerance reported varies from a gradual decrease over weeks or months ([12,31]; also Walsh, Leber and Bowman, in preparation) to a complete absence of tolerance [23,30].

One possible reason for the discrepancy between clinical and experimental findings is that the types of pain involved are different. Most animal pain tests involve measuring the threshold at which a stimulus becomes noxious, and depend on the animal making a withdrawal response, usually before tissue damage occurs [14,18]. This type of pain has been called "first pain" [24], "sharp, pricking pain" [5], or "phasic pain" [9]. It is well localized and relatively insensitive to opiates [7,17]. However, in clinical medicine narcotics are normally used for the pain that follows tissue injury. This pain has been described as "true pain" [5] or "tonic pain" [9] and is dull, continuous and poorly localized [4, 7, 9, 17].

Recently Dubuisson and Dennis [10] developed a pain test for rats which mimics some features of post-injury pain. In the Formalin test a small amount of dilute Formalin is injected subcutaneously into the animal's forepaw and the animal's behavioral response is rated during the following hour. Experimenters who have experienced the pain describe it as poorly localized, burning and throbbing. Results obtained with this test confirm that the distinction between the two types of pain is a real one. Lesions and antiserotonergic drugs, which have been shown to block morphine analgesia in the tail-flick and hot-plate tests [26, 27, 32], potentiate or do not change the effects of morphine in the Formalin test [1,8].

We now report that in the Formalin test, tolerance to morphine analgesia is very slow or non-existent and that conditioned- or behavioral-tolerance does not occur. These findings further support the view [9] that different pain tests measure different types of pain with different mechanisms of morphine analgesia.

METHOD

The procedure is similar to that used previously to demonstrate conditioned tolerance [2, 3, 28]. The animals were assigned to one of 6 groups. The test cage-morphine (TC-M, n=8) and the test cage-saline (TC-S, n=8) groups were injected each day for 5 days with morphine or saline in the test room and immediately placed in the test cage for 30 min. The home cage-morphine (HC-M, n=8) and home cage-saline (HC-S, n=8) groups were injected in the colony room each day for 5 days and returned to their home cages immediately. A control group (S, n=8) was injected with saline on the test day and tested with the Formalin test to provide a baseline pain level with which to compare morphine analgesia. To determine if longer treatment with morphine would result in more tolerance to its analgesic effect, an additional group was injected with morphine in the colony room for 15 days and then treated the same way as the TC-M group for 5 more days; these rats comprise the 20 D-M group (n=10).

All rats in the morphine groups received 7 mg/kg of morphine sulphate dissolved in normal saline, 7 mg/ml. This solution was injected subcutaneously. The test cage consisted of a clear Plexiglas (30×30×30 cm) chamber. A mirror was mounted at 45° angle below the floor to allow an unobstructed view of the paws.

On the day of the Formalin test in the test room, morphine was administered to all animals except those in the control group (S) which received saline. The animals were then placed in the test cage. Ten min later, 0.03 ml of 2.5% Formalin was injected subcutaneously into the dorsal surface of one forepaw of each rat. Pain rating began immediately and continued for 50 min. Pain was rated by recording only the amount of time the sore paw was elevated with, at most, the nails touching the floor. This evaluates the degree of tenderness and hyperaesthesia. Chewing of either the Formalin-injected paw or another extremity was also recorded. A continuous record of pain response was obtained by the observer by manipulating a switch which produced a DC signal that was amplified and recorded on a Grass polygraph. The paper records were later scored to obtain average pain responses for each animal.

This pain scale was modified from that developed by Dubuisson and Dennis [10] because pilot experiments indicated that rats treated repeatedly with morphine have a tendency to persistently chew some part of their bodies, even when not injected with Formalin. This behavior distorts their pain scoring scale which is an average score based on the relative amounts of time spent chewing, elevating, and favoring the injected paw.

RESULTS

Figure 1 shows the average amount of time the Formalin injected paw was elevated in the 6 groups of rats. The pain scores of the saline control group had a profile similar to that described by Dubuisson and Dennis [10] using the full pain rating scale. The pain levels were high during the first few minutes and then decreased for about 10 min before rising to a higher level for about 1 hr. This pattern of pain has also been described by experimenters who have experienced a Formalin injection.

It is evident in Fig. 1 that morphine produced a significant reduction in pain levels in all groups. Because the variance

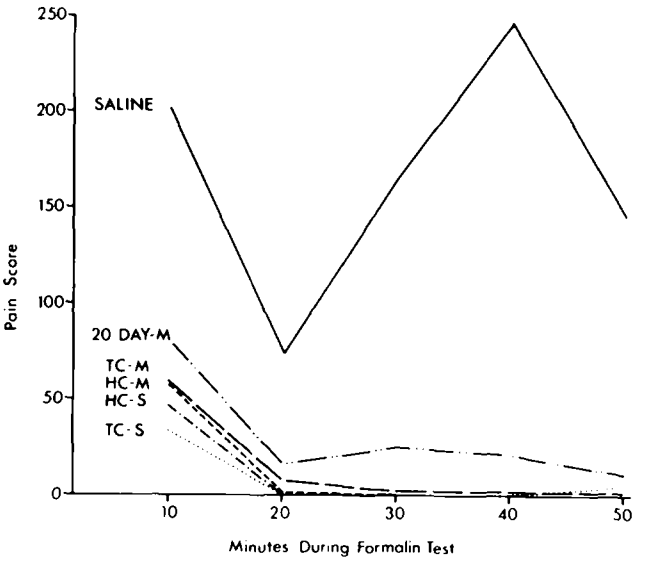

FIG. 1. Ordinate shows the mean number of seconds in each ten-min block that rats held the Formalin-injected paw elevated, with, at most, the nails touching the floor. The saline curve represents the normal course of pain in undrugged rats. The other curves show the effect of morphine (7 mg/kg) given for the first time (HC-S, TC-S) or after 5 (HC-M, TC-M) or 20 (20 D-M) daily morphine injections.

estimates were correlated with the means, an analysis of variance (BMD P2V) was performed on square-root transformed scores. There was a significant effect of group, $F(5,44)=35.36, p<0.0001$, of time, $F(4,176)=39.0, p<0.0001$, and of group-by-time interaction, $F(20,176)=3.70, p<0.0001$. A Newman-Keuls analysis showed that the interaction was due to the differing time course of the pain for the control group and the morphine-treated groups. All morphine-treated groups showed a similar pattern and there was no effect of the environment in which morphine was administered (p's all>0.05). Indeed, there was little sign of tolerance even in the group which received morphine injections for 20 days, including 5 days of exposure to the testing environment. While the mean pain scores were slightly higher for this group (see Fig. 1) than the other morphine treated groups, this difference did not reach significance at any time during the test (p's>0.05) nor when the pain scores were averaged for the full 50 min of the test (p's>0.05).

Figure 2 shows the average amount of time the animals spent chewing and grooming the sore paw or some other area of the body. It is clear that repeated injections of morphine greatly increased the tendency to worry some area of the body. Analysis of variance showed a significant effect of group, $F(5,44)=3.84, p<0.006$. However, analysis of variance between the three groups pre-exposed to morphine (HC-M, TC-M and 20 D-M) showed no significant differences between groups, $F(2,23)=0.10, p<0.9$, or between the tendency to chew the Formalin paw or another area, $F(2,23)=1.42, p<0.2$. The Moses-test of extreme reaction revealed another property of the paw-chewing. The amount of chewing of the Formalin paw in the morphine-treated groups lay outside the range of the saline control group and

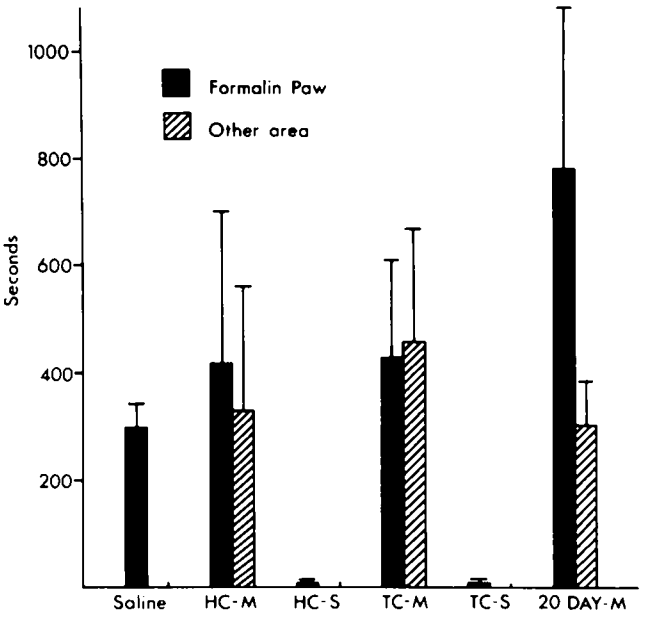

FIG. 2. The mean number of seconds in the 50-min test period that rats spent chewing or worrying the Formalin-injected paw or some other area of their bodies. Vertical lines show standard errors of the means.

was either greater or less than the saline animals (when $h=0$, $p=0.000427$). In other words, after chronic morphine treatment, the animals tend to chew the Formalin-injected paw much more or much less than the saline group. Moreover, when they chew the Formalin paw less, they tend to focus chewing on some other part of the body. This finding supports the use of the modified pain rating scale in studies of chronically administered morphine. Since morphine is known to produce flushing and itching in man [11], the paw-chewing behavior may represent a similar action in the rat.

DISCUSSION

The present data indicate that in the Formalin test, situation-specific tolerance does not occur. This is consistent with the human clinical literature in which there are no reports of a dramatic and situation-specific decrease in the analgesic potency of narcotic drugs during the first few days of treatment.

More surprising is the apparent overall absence of tolerance even in the group of rats that received morphine for 20 days. The 7 mg/kg subcutaneous dose of morphine used in the experiment initially produces good analgesia in both the tail-flick [25] and hot-plate tests [3]. In the hot-plate test, strong conditioned tolerance occurs in 4 to 6 days [2, 3, 16, 28, 29]. In the tail-flick test, the analgesic potency of 15 mg/kg IP decreases to about 1/4 in 5 days [25]. Fifteen mg/kg IP is approximately equal to 7 mg/kg subcutaneously [21].

It might be argued that the hyperactivity which develops with tolerance to morphine interferes with the pain rating so that pain levels, and therefore tolerance, were underestimated. However, in another study in which activity and pain were simultaneously monitored [1], the two measures were uncorrelated over a range of doses and durations of morphine administration. Furthermore the locomotor effects of morphine are strongly situation-dependent (Abbott, in preparation) and would therefore be expected to contribute to differences between the test cage-morphine (TC-M) and home cage-morphine (HC-M) groups. The data showed no evidence of such differences.

It should be noted that narcotic drugs have multiple effects which vary considerably in the rate at which tolerance develops. In man, tolerance develops rapidly to the respiratory depressant [15] and sedative effects [12,30] of morphine. Similarly, Mucha *et al.* [25] report that in rats the dose of morphine can be rapidly escalated to four or five times the lethal dose. Tolerance to emetic and antitussive effects are much slower [12]. At the other end of the scale, the stimulant effects of morphine on intracranial self-stimulation [13] and the threshold at which a rat can discriminate narcotics from saline [6] do not appear to change over time. Thus, a priori, there is no reason to expect any particular rate or degree of tolerance to any given effect of morphine. At the same time, the fact that analgesia in the Formalin test shows weak or slow tolerance, while tolerance to morphine analgesia is rapid and profound in the commonly used withdrawal tests, reinforces other evidence [1,8] that analgesia as measured in the two types of tests depends on different neural mechanisms.

The human clinical literature does not provide a basis for predicting the pattern of tolerance to be expected in a good animal model of analgesia, since examples can be found to support every position. At one end of the scale, Houde *et al.* [19] report a 30–50% decrease in the potency of morphine and methadone in 7 days in 13 cancer patients but they provide no information on the degree of pain control originally achieved, the changes in the disease processes, or on the variances in the data. More recent clinical studies, in which the changes in pain-producing disease were estimated found no experience of tolerance [23,30]. Furthermore, Twycross [31] has argued that in patients whose condition is stable, escalation of the dose of analgesic is rare, implying that tolerance is minimal. On the other hand, Walsh *et al.* (in preparation) do find definite evidence of tolerance in a large series of terminal cancer patients. The need for increased doses of narcotics is, however, much smaller than that predicted from animal studies using heat pain or by Isbell *et al.* [20] using heat pain in post-addict human subjects.

The Formalin test, in which morphine tolerance is weak, may therefore be a useful model of analgesia in clinical pain. At the very least, our findings indicate that the apparent tolerance depends on the method used to evaluate pain and, in the light of the contradicting clinical literature, suggest that the significance of the rapid tolerance seen with reflex withdrawal tests needs to be re-examined.

ACKNOWLEDGEMENTS

This research was supported by Natural Sciences and Engineering Research Council of Canada Grant A6303 to K. Franklin and Grant A7891 to R. Melzack.

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