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The mouse tail-flick method for evaluation of narcotic antagonist sustained release

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Summary

The objective of this research was to evaluate the mouse tail-flick test for analgesia as a method for screening sustained release narcotic antagonist delivery systems. Control experiments indicated that neither spontaneous movements of the mouse tail nor aging during sustained release studies has any appreciable influence on the measurements. However, testing the mice on a second occasion resulted in more variability in response to the agonist morphine, thus precluding use of a mouse for more than one measurement. Using naltrexone as the narcotic antagonist, it is shown that appropriate use of controls permits conclusions with regard to the physicochemical mechanism responsible for sustained release. Although this pharmacodynamic method does not measure in vivo release rates per se, data from several sustained release systems indicate that this test can be used to assess duration of prolonged release.

Introduction

Narcotic antagonists are a viable therapy alternative to analgesic maintenance for the treatment of opiate addiction. One of the more promising narcotic antagonists that has been evaluated in humans is naltrexone (Julius and Renault, 1976). Naltrexone, however, has a relatively short duration of action in humans of 48 h with a 70 mg oral dose (Volavka et al., 1976). This is not felt to be a long enough interval between doses to aid the addict in becoming dissociated from drug-taking behavior. Thus, various sustained release systems, both physical and chemical, have been prepared at a variety of industrial and academic institutions as part of a research program sponsored by the National Institute on Drug Abuse (Willette, 1975; Willette and Barnett, 1981). The overall strategy for animal evaluation of these systems has been described (Dewey, 1981). This strategy included an initial pharmacologic screening method, the mouse tail-flick assay (Dewey et al., 1969, 1970) for analgesia, as modified to permit quantitation of analgesic antagonism.

This report describes the apparatus and methodology used to quantitate duration of analgesic antagonism from a variety of sustained release

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narcotic antagonist preparations as well as experiments carried out to document the validity and limitations of the method.

Materials and Methods

Apparatus and tail-flick procedure

The mouse tail-flick apparatus (Fig. 1) was designed to permit application of a reproducible temperature to a small portion of the mouse tail and to prevent general heating of the apparatus with time. The testing unit consists of an aluminum block having a heating coil recessed 1/4 inch in a slit in the block and having a circulating water-cooling system. The testing unit is mounted on a wood supporting base and the hot wire (Fig. 1) is connected to a variable transformer. Mice are restrained in a plastic tube with a notched stopper which permits exposure and manipulation of the tail (Kaplun et al., 1972). The tail is placed across the slit above the heating coil and temperature can be carefully adjusted using a variable transformer and a pyrometer placed near the tail surface. It was found that a temperature of 50 °C produces a 5-s response time in untreated mice. Morphine sulfate was administered intraperitoneally at a wide range of doses to construct a log dose–response curve under these experimental conditions. The 100% analgesic response of 15 s was chosen to avoid tail injury. The ED_{80} (13 s response time) was used as a reproducible analgesic (agonist) response that served as a reference point for other mice in which the narcotic antagonist was administered at some time prior to administration of the ED_{80} morphine dose. The degree of antagonism of a narcotic antagonist to morphine was calculated according to the following formula:

$$\% \ antagonism = \frac{\left(\begin{matrix}mean\ agonist\\response\ time\end{matrix}\right) - \left(\begin{matrix}mean\ response\ time,\\agonist\ with\ antagonist\end{matrix}\right)}{\left(\begin{matrix}mean\ agonist\\response\ time\end{matrix}\right) - \left(\begin{matrix}mean\ vehicle\ control\\response\ time\end{matrix}\right)} \times 100$$

Mice were tested in a blind manner and values reported are each the mean of 5 mice except where noted. Untreated and morphine ED_{80}-treated mice were also tested each day in a blind manner as controls. Mean initial weight of the mice was approximately 20 g (CV < 5%).

Male, albino, CF-1 mice (Carworth Farms) were used for the control studies in Table 2 and Fig. 2 and for delivery systems 1 and 2 (Table 1). The ED_{80} of morphine sulfate in these mice was 3.3 mg/kg administered i.p. at 60 min before the response measurement (time of peak response). When the supply of these mice became unreliable, Cox Swiss Webster mice (Lab Supply, Elkhart, IN) were used for the control study in Table 3 and for delivery systems 3–5. The ED_{80} for morphine in these mice was 7.1 mg/kg administered i.p. 45 min before the response measurement.

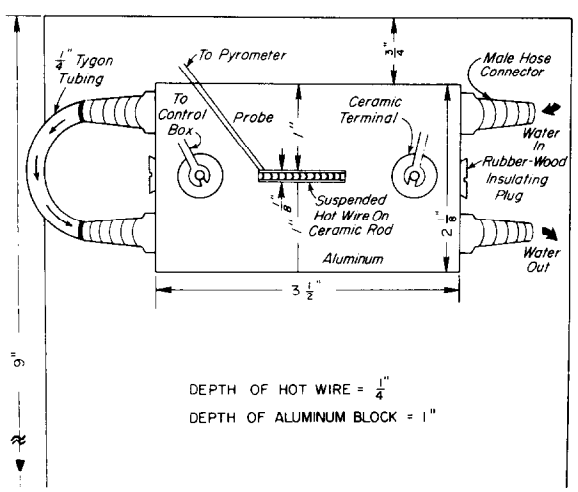

Fig. 1. Mouse tail-flick apparatus, top view.

Control studies

Control studies were designed to examine several aspects of the tail-flick testing procedure. In the first experiment the response time of the mice was measured by carrying out the test described, with heat (50 °C) and without heat (room temperature) applied to the tail both for morphine-treated mice and saline-treated controls. This permitted the assessment of extraneous experimental variables, other than the heat applied to the tail, on the measured response times. The second experiment was designed to determine the influence of aging on the tail-flick response time. Mice were tested (untreated and morphine ED_{80}

TABLE 1

Characteristics of naltrexone sustained release systems

Delivery system description	Constituents prolonging release	Naltrexone content (% of total weight)	Particle size	Vehicle and concentration (mg naltrexone equiv./ml)	Dose (mg naltrexone equiv./kg)	Route of injection	Needle gauge	References
No. 1								
Granules of naltrexone–polymer physical mixture	95% Polylactate 5% Citroflex-4	35	25–35 mesh	7% sodium carboxy-methyl cellulose (1.4 mg/ml)	38.8	s.c. (lower back)	12	(Yolles, 1975; Yolles et al., 1975)
No. 2								
Amorphous naltrexone salt–metal complex	Naltrexone gallotannate–zinc complex	23–27	Suspended powder	2% aluminum monostearate in peanut oil (10 mg/ml)	40	i.m. (thigh)	18	(Gray, 1974; Gray and Robinson, 1974)
No. 3								
Spheres of naltrexone–polymer physical mixture	75% L(+)-lactate 25% glycolate copolymer	50	1.5 mm dia. spheres	None	50 or 100	s.c. implant (upper back)	12	(Schwope et al., 1975a and b; Sadek 1976)
No. 4								
Microencapsulated naltrexone pamoate	DL-polylactate polymer	26–29	63–106 μm	2% aluminum monostearate in peanut oil (6–7 mg/ml)	33–37	s.c. (caudal to left front leg)	20	(Thies, 1976)
No. 5								
Microencapsulated naltrexone base	DL-polylactate polymer	20 or 44	63–106 μm	0.9% NaCl with added surfactant *or* peanut oil (7–9 mg/ml)	28–35	s.c. (caudal to left front leg)	20	(Thies, 1976)

controls) at time intervals of 1–50 days after selection of the entire group of mice for the study. Selection was based on initial body weight being within 15% of the mean of 20 g. In the third experiment the objective was to determine if mice could be tested more than once for morphine analgesia. A group of 60 mice was administered morphine and evaluated for tail-flick response time. These 60 mice were then divided into 3 groups and evaluated again, each group at a different time subsequent to the first test (7, 14 and 24 days).

Sustained release systems

A summary of the several delivery systems that were analyzed is presented in Table 1. Details concerning the fabrication and characteristics of these systems are included in the references cited. In general, 4 of the systems could be administered by injection of a suspension. System no. 3 was implanted using a plunger to push the sphere through the 12-gauge needle. When large bore needles (12-gauge) were used, a single suture was necessary to prevent leakage.

The sustained release naltrexone delivery systems were tested in vivo by administration to mice and evaluation of the tail-flick response to the standard ED_{80} dose of morphine sulfate. The sustained release system was administered at varying times prior to tail-flick testing with the range being from a few days to more than one month. A percent analgesic antagonism–time profile was constructed to determine: (1) the duration of antagonist response due to the naltrexone delivery system; and (2) the effect of the vehicle and delivery system constituents on duration of antagonism.

Results and Discussion

The possible influence of unrelated environmental stimuli on the results of the tail-flick test were assessed by comparing the response time without a heat stimulus to that obtained with a heat stimulus (Table 2). The response time without stimulus was considerably longer for both untreated and morphine-treated mice. This indicates that the important determinants of the tail-flick response time are the heat stimulus and the effect of morphine analgesia on response to that stimulus, and not spontaneous movements of the mouse tail.

TABLE 2

Comparison of tail-flick response time with and without a heat stimulus

Pretreatment [a]	Response time (s)	
	Without stimulus	With stimulus
None	24.6 ± 9.4 [b]	5.04 ± 0.26 [b]
Morphine	32.8 ± 12.1 [c]	13.0 [d]

[a] Morphine ED_{80} dose of 3.3 mg/kg i.p. 1 h before testing.
[b] Mean of 25 determinations in a cross-over study ± S.D. These mean values are significantly different ($P < 0.05$, t-test).
[c] Mean of 18 determinations ± S.D. A cut-off time of 45 s was utilized and this value was used for the 6 mice that did not respond within 45 s.
[d] Usual response time with this dose of morphine in other mice. A cross-over was not used in the morphine mice because of the tolerance which may develop after a single dose.

The stability of the tail-flick response over time was tested in a group of 130 mice, with each mouse being tested once. The time of testing was varied in different mice over a time period of 50 days from initiation of the study in order to include the expected duration of release in sustained release narcotic antagonist studies. Results shown in Table 3 indicate excellent stability of the tail-flick test over 50 days, despite considerable aging of the mice.

The possibility of using each mouse as its own control was also investigated. This would require being able to perform the tail-flick test a second time while maintaining good stability of the test over time. Results of testing mice for morphine response time on a second occasion after either 7, 14 or 24 days are shown in Fig. 2. It is clear from these results that the tail-flick test has more variability associated with the second test at either 7 or 14 days, possibly due to a memory factor. However, there were no significant differences between any of the mean values in Fig. 2 ($P > 0.05$, t-test). Comparison of the results in Fig. 2 with the excellent stability when the mice are tested only once (Table 3) indicates that the latter is the

TABLE 3

Mouse tail-flick response as a function of time after initiation of study

Time (day)	Response time (s) [a]	
	Untreated controls	Morphine (ED_{80})-treated controls
1	4.8±1.0	12.6±0.6
4	4.9±1.0	13.9±1.1
6	4.9±0.7	13.5±1.5
7	5.3±0.7	13.5±0.7
8	5.3±0.4	13.3±0.7
10	5.0±0.3	12.6±1.1
18	5.4±0.9	12.7±0.8
21	4.8±0.5	12.5±0.4
25	4.9±1.1	13.0±0.4
32	5.3±0.9	13.1±0.4
35	4.6±0.7	13.4±0.7
40	5.0±0.6	13.0±0.3
50	5.0±0.6	13.0±0.3

[a] Mean of 5 mice ±S.D.

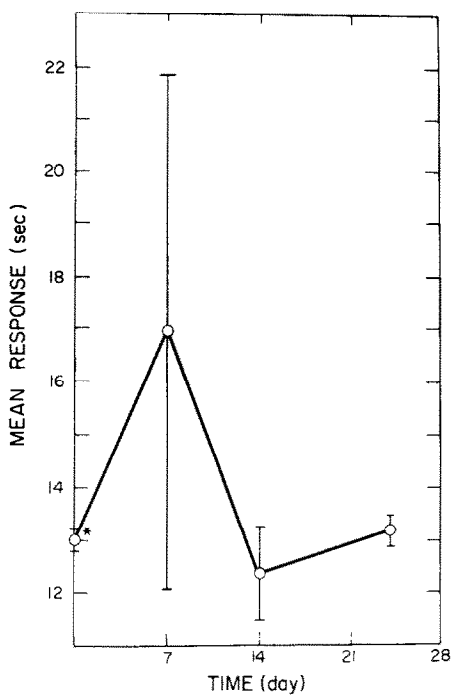

Fig. 2. Effect of a second dose of morphine on mouse tail-flick response time. Asterisk indicates results for the first dose in 60 mice and other points are each for 20 of these mice tested a second time. Morphine administration as in Table 1 and verticle bars indicate S.D.

preferred approach. Thus, in all evaluations of sustained release delivery systems mice were tested only once.

The mouse tail-flick methodology has been applied as an initial screening test for prolonged release of narcotic antagonists in our laboratory (Table 1) and by others (Willette and Barnett, 1981). Data in Table 1 indicate that the method can be applied to delivery systems with a wide variety of physical and chemical characteristics. Often the most difficult experimental challenge is finding an appropriate suspending vehicle and/or route of administration. When desired, the injection site may be examined for gross pathology subsequent to completion of the tail-flick procedure.

Use of appropriate controls in the tail-flick test permits an evaluation of the physicochemical mechanism for prolonging release from a narcotic antagonist delivery system. In Fig. 3 the duration of at least 50% antagonism for > 10 days could be due to the expected mechanism of slow release of naltrexone from the polymer matrix or to several other possible factors. Experiments without drug indicated no effect of vehicle or polymer administration on the tail-flick test results. Comparison of sustained release studies with control studies in which either drug, drug plus suspension vehicle, or drug plus vehicle plus blank polymer was administered permits the conclusion that release of naltrexone from the polymer matrix is the physicochemical mechanism responsible for the prolonged duration of response (Fig. 3). A small degree of prolongation of antagonist response may also be attributed to the CMC vehicle itself but not to the presence of blank polymer (Fig. 3). Similar types of controls were included in the evaluation of the other delivery systems.

One limitation of the tail-flick test when used for measuring analgesic antagonism is the "ceiling" that occurs at high percent antagonism values (80–100%). An example of this, that was characteristic of results from several other delivery systems, is shown for the polymer delivery system in Fig. 3 over the first 5 days. When levels of the competitive antagonist are sufficiently high to overcome the morphine effect of the constant ED_{80} dose (used for stability of the test), complete

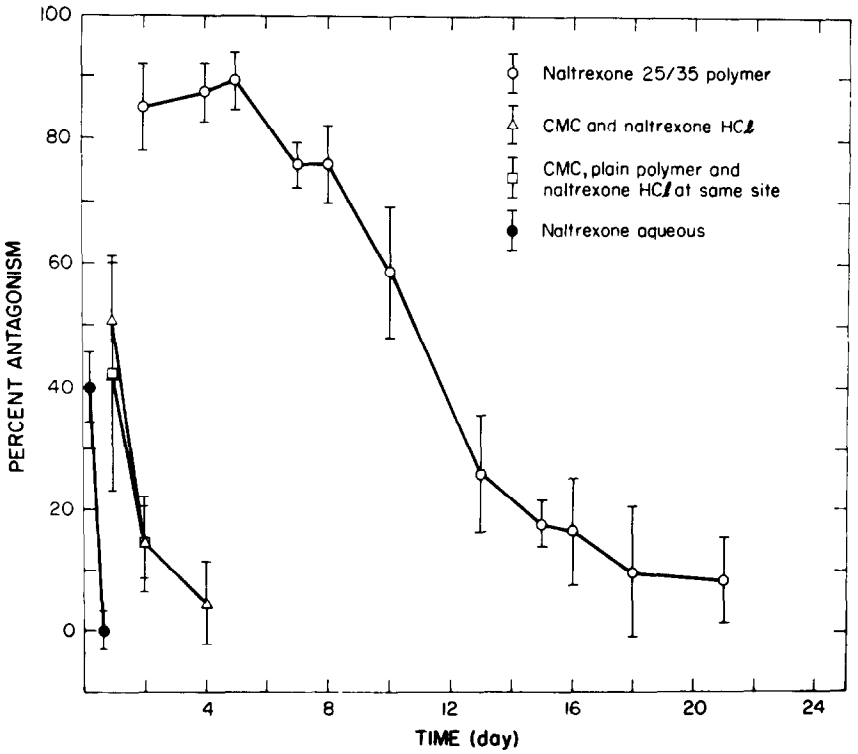

Fig. 3. Percent analgesic antagonism–time profile for naltrexone in mice showing results for naltrexone-HCl aqueous solution, naltrexone-HCl plus the 7% sodium carboxymethylcellulose (CMC) vehicle, naltrexone-HCl plus the CMC vehicle and the blank polymer delivery system, and the 25/35 mesh polymeric sustained release naltrexone delivery system (No. 1, Table 1) suspended in the CMC vehicle. The dose of naltrexone in non-sustained release experiments was the same as for the sustained release study. Bars indicate standard deviations and differences between the sustained release system and controls were statistically significant ($P < 0.05$, t-test) at times when both were measured.

antagonism results. Thus, in this range of high narcotic antagonist levels, the tail-flick test will not be responsive to changes in antagonist body levels or release rates. However, the *duration* effective release rates can be assessed and ranged from about 16 days (Fig. 3) to about 42 days (delivery system no. 4, Table 1).

Overall then, the mouse tail-flick test for analgesic antagonism can be used to test hypotheses related to the physicochemical mechanism responsible for prolonging release of a narcotic antagonist and to determine the influence of formulation variables on the duration of sustained release. Limitations of the methodology include the inability to quantitate release rates with this technique and the difficulty of administering intramuscular injections and of administering delivery systems scaled to a 70 kg human size in such a small animal. In our laboratory the method provided a cost-effective initial screening test for duration of sustained release that was supplemented with quantitative determinations of in vivo release rates for promising naltrexone delivery systems (MacGregor et al., 1983; Reuning et al., 1983).

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