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Research Article

A New Model to Assess Analgesic Activity: Pain-Induced Functional Impairment in the Rat (PIFIR)

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ABSTRACT A new experimental model to assess analgesic activity of both analgesic and nonsteroidal antiinflammatory drugs is described. It uses the unilateral intra-articular knee injection of an uric acid suspension in mineral oil to produce acute inflammation, pain, and functional motor impairment. The model, named "pain-induced functional impairment in the rat" (PIFIR) assesses analgesic activity by measuring the capacity to walk with the injured extremity. The procedure determines both the potencies of analgesic drugs and the time course of the effect. Analgesia of selected reference agents was followed for 4 h and the effect versus time curves were constructed. The area under the curve (effect versus time), an expression of the overall activity during the observation period, increased in a dose-dependent manner. The area under the curve, E_{max}, T_{Emax}, and ED_{50} of reference agents tested are reported. The PIFIR procedure was sensitive to opiate and nonopiate analgetics (nonsteroidal antiinflammatory drugs) and possibly steroidal antiinflammatory drugs. These characteristics make it suitable for screening purposes.

Key Words: pain model, analgesia, PIFIR, opioids, nonsteroidal antiinflammatory drugs, rat

INTRODUCTION

Understanding of the mechanisms of analgesia and the development of novel analgesic agents depends to a major extent on the available animal methods for the quantitative determination of pain. The majority of the traditional assays for analgesic activity measure transient rather than continuous pain. These models vary widely in the type of noxious stimuli applied. Some of these use thermal stimuli [D'Amour and Smith, **1941;** Woolfe and McDonald, 1944], while others use mechanical [Bianchi and Franceschini, **1954;** Collier et al., **1961;** Randall and Selitto, 1957], electrical [Turker and Turker, **1970;** Ayhan et al., **19831,** and chemical stimulation [Siegmund et al.. 1957].

The relevance of phasic endpoints yielded by acute stimuli to clinical pain has been questioned, as the responses in the aforementioned methods ar, ar_ tificial noxious stimuli [Pircio et al., 1975]. A better understanding of the physiological processes involved in nociception during tonic pain states is required if novel analgesics are to be properly evaluated [Wheeler-Aceto and Cowan, 1991].

There are several models of persistent pain. One is adjuvant-induced polyarthritis in the rat [Pearson, 1956]. This procedure has been well characterized [De Castro Costa et al., 1981] and has proven to be useful for the assay of analgesic agents [Pircio et al., 1975]. However, the induction of polyarthritis results in an extended systemic involvement and therefore observed responses cannot be confidently considered as due to nociception alone [Wheeler-Aceto and Cowan, 1991].

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Alternative methods have been described, such as intra-articular or subcutaneous injections of a variety of substances in a hind limb [Faires and McCarty, 1962; Pardo and Rodriguez, 1966; Van Arman et al., 1970; Okuda et al., 1984; Wheeler-Aceto and Cowan, 1991]. These procedures have been useful for both the assay of analgesic drugs and the characterization of the inflammatory process.

To adequately evaluate the potential efficacy of an analgesic agent, it is necessary to determine not only the analgesic efficacy at a fixed time but the duration of the effect [Mattok, 1988]. Methods that allow the determination of the time course of analgesia will facilitate characterization of the mechanisms of action of analgesic drugs and pharmacokinetic/pharmacodynamic studies.

Pardo and Rodriguez [1966] developed a method in dog which allowed them to quantitatively follow the analgesic action with time. Another procedure developed by Van Arman et al. [1970] allowed a quantitative estimation of analgesia, but all measurements were taken at fixed time. Okuda et al. [1984] reported a modified version of this procedure using cats that allowed them to follow the time course of morphine in a quantitatively fashion. These three procedures, although they are suitable for the determination of analgesia over time, have the disadvantage that animals must be submitted to lengthy training periods to obtain reliable results. Nonetheless these procedures are useful in studying the physiological bases of pain and analgesia but are impractical for screening purposes.

In the present study, we report a method of pain-induced functional impairment in the rat (PIFIR). This procedure determines the potency and duration of analgesic action produced by various classes of compounds by measuring their effect on inflammation-induced tonic pain and permits the quantitative evaluation of the kinetics of the analgesic effect.

MATERIALS AND METHODS

Animals

Female Wistar rats, 180–220 g, from our own breeding facilities [Crl: (WI)BR] were used in this study. Twelve hours before experiments, food was withheld with free access to water. All procedures performed followed the recommendations of The Committee for Research and Ethical Issues of the International Association for the Study of Pain [1980].

Measurement of Analgesic Activity

The animals were anesthetized with ether in an anesthesia chamber (glass dryer Pirex saturated with ether vapors). Pain was induced by an intra-articular injection of 0.05 ml of 30% uric acid suspended in mineral oil in the knee joint of the right hind limb. The suspension was prepared by grinding 3.0 g of uric acid with 10 ml of mineral oil in a glass mortar and pestle (Pirex). The intra-articular injection was performed through the patellar ligament using a 1 ml glass syringe (Becton, Dickinson LTDA, Brazil) with a 24 gauge needle of 5 mm. Immediately, an electrode was attached to each hind paw between the plantar pads. Rats were allowed to recover from anaesthesia and were then placed on a stainless steel cylinder of 30 cm diameter. The cylinder was rotated at 4 RPM, forcing the rats to walk. Training periods were not necessary because the rats learning in the first minutes. The variable measured was the time of contact between each of the rat's hind paws and the cylinder. When the electrode placed on the animal's paw made contact with the cylinder floor, a circuit was closed and the time that the circuit remained closed was recorded. The cylinder was rotated for 2 min periods, during which time recordings were made with 30 min rest periods between recordings. In all subsequent experiments, analgesic agents were administered 2.5 h after uric acid injection. Thus, this time was considered as time zero for measurements of analgesic effect. Drugs were given at this time and the time of contact was measured every 30 min for 4 h. All experiments were performed between 7:00 am and 14:00. The animals were then sacrificed.

Instrumentation

The rotary walking device consisted of a hollow cylinder made of stainless steel of 30 cm diameter and 60 cm long. The cylinder was divided into six individual tracks with annular pieces of stainless steel of 48 cm of external diameter. Each track was covered with stainless steel wire mesh (20 mesh per inch) to provide a nonslip surface for the animals. This arrangement allowed the study of six animals at the time. An electrical motor (Westinghouse Elec. Corp. Model 309P384-A) with electronic control rotated the cylinder at a constant speed of 4 RPM.

The time of contact of each hind paw was recorded by plantar electrodes. Each electrode consisted of a drop of silver solder in a disk-like form placed at the end of a 26 gauge insulated copper wire. The metallic disk was glued to a piece of rubber film that was adhered to the hind paw with cyano-acrylic adhesive to prevent direct contact between the metal and the skin. The other end of the copper wire was connected to the time counting device. The schematic diagram used for counting the time of contact between the electrodes and the cylinder floor is shown

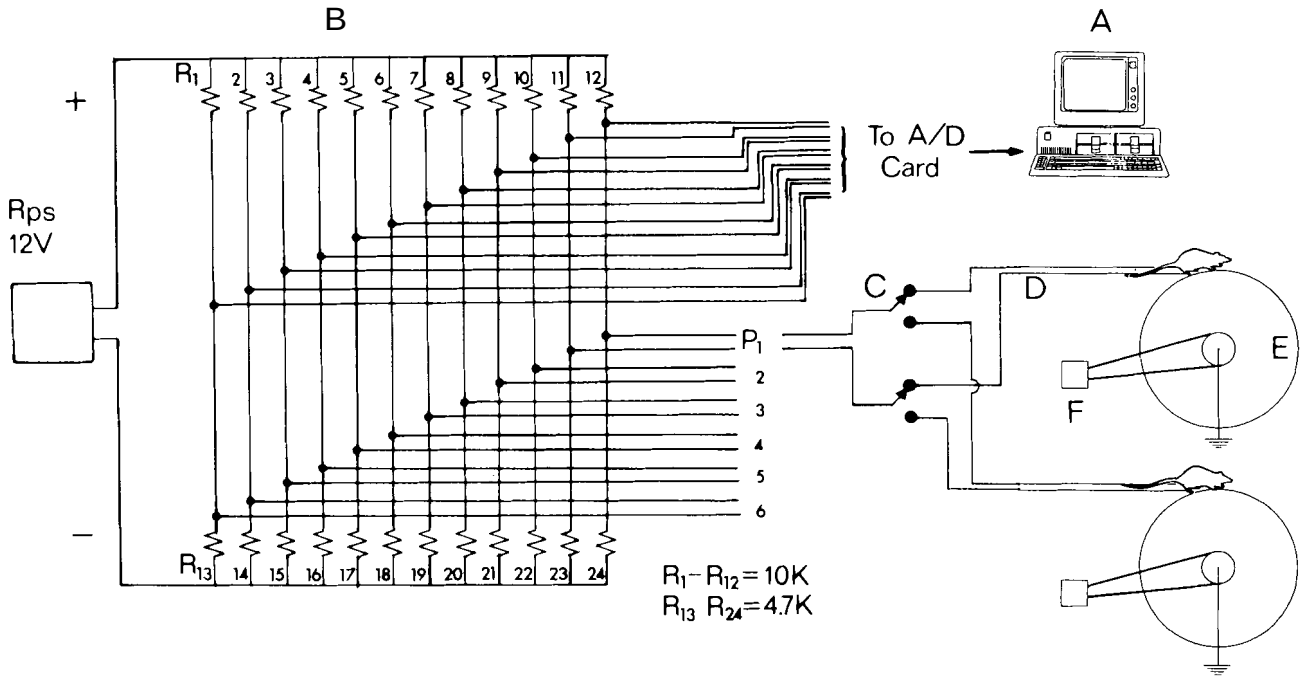

Figure 1. Schematic diagram of the system used for recording of the time of contact between the rat's hind paws and the cylinder floor. A: Time counting device, B: switch box, C: switches, D: electrodes, E: rotary walking stainless steel cylinder, F: rotating motor drive.

in Figure 1. An Apple@ II+ computer equipped with a Mountain@ AID board and a switch box was used to collect data by means of a Basic software routine (Fig. 2).

Drugs

Uric acid was purchased from Sigma Chemical Co. (St. Louis, MO), Dipyrone sodium (Hoechst), morphine hydrochloride (Mexican Secretariat of Health), pentazocine hydrochloride (Winthrop Products), d-propoxyphene hydrochloride (ElinLilly), and hydrocortisone 21-phosphate (Sigma Chemical Co., St. Louis, MO) were dissolved in distilled water. Acetylsalicylic acid (Miles) and acetaminophen (Sigma Chemical Co., St. Louis, MO) were suspended in 0.5% carboxymethyl cellulose. Indomethacin (Merck, Sharp and Dohme) was dissolved in a sodium bicarbonate solution. Adequate controls were performed with each of the used vehicles. Doses of each agent are referred to the particular salt.

Data Analysis

Data are expressed as the functionality index (FI). This is the time of contact of the injected limb divided by the time of contact of the control left limb multiplied by 100.

The maximal observed effect (E_{max}) can be expressed in terms of FI and the time required to reach this response (T_{Emax}), as shown in Table 1. These parameters reflect the potency and the rate of onset of drug action by a given route. Furthermore, since the time course of the effect has been followed, it is possible to use the cumulative analgesic effect during the whole observation period as the area under the curve (AUC) in a similar manner to that used in pharmacokinetics to express the amount of drug absorbed [Gibaldi and Perrier, 1975].

Since the AUC value represents the integrated analgesic effect during the observation period and thus includes both the maximal response and the duration of action, this expression was preferred for the construction of dose-response curves. The maximal possible AUC value that could be achieved under the experimental conditions was 375 area units (% . h). This is due to the fact that the maximal FI is 100% and the observation period consisted of 4 h with the first determination being performed at 0.5 h. Considering that the FI reached 100% in 0.5 h and remained at this value until the hour 4, the AUC obtained is 375 units (obtained with the trapezoidal rule and taking in account the area of the first triangle).

All values in the text and figures are mean of at least six animals ± SE. The area under the curve

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PROGRAM IN BASIC TO GATHER DATA IN THIS MODEL

 10 TEXT: HOME: CLEAR: PRINT "<<<PIFIR>>>": PRINT
 20 INPUT "NAME OF DATA FILE? (RETURN = NONE) ":NO$
 30 IF NOS = "" THEN 60
 40 N = 451: INPUT "NUMBER OF READINGS? (451 = 2 MINUTES) "; R$
 50 IF R$ < > "" THEN N = VAL (R$)
 60 DIM D(12),V(12),C(12)
 70 REM **** SET UP ADDRESS VECTOR ****
 80 FOR I = 1 TO 12
 90 D(I) = 49315 + I:V(I) = 0: NEXT
100 C = D(1): FOR I = 1 TO 11:D(I) = D(I+1): NEXT:D(12) = C
110 REM **** CLEAN UP READING ****
120 FOR I = 1 TO 12:C = PEEK (D(I)): NEXT
130 IF NO$ = "" THEN 420
140 REM **** READING LOOP ********************
150 FOR I = 1 TO N: FOR J = 1 TO 12
160 C = PEEK (D(J))
170 IF C < 175 THEN V(J) = V(J) + 1
180 IF C > 175 THEN C(J) = C(J) + 1
190 NEXT : NEXT : PRINT CHR$(7)
200 REM **** DISPLAY VALUES ON SCREEN ********
210 HOME
220 VTAB 1: PRINT "CONTROL        INJECTED"
230 FOR J = 2 TO 12 STEP 2: VTAB J: PRINT V(J), V(J -1): NEXT
240 IF NOS = "" THEN END
250 INPUT "SAVE DATA? (Y/N) ";R$: IF R$ = "N" THEN 340
260 PRINT CHR$(4) "OPEN "NO$: ",D2"
270 PRINT CHR$(4) "WRITE "NO$
280 PRINT CHR$(4) "CLOSE "NOS
290 PRINT CHR$(4) "APPEND "NOS
300 PRINT CHR$(4] "WRITE "NO$
310 FOR I = 1 TO 12
320 PRINT V(I): NEXT
330 PRINT CHR$(4) "CLOSE "NOS
340 PRINT: PRINT: INPUT "ANOTHER READING? (Y/N) "; R$
350 IF R$ = "N" THEN 392
360 FOR I = 1 TO 12
370 V(I) = O:C(I) = 0: NEXT
380 IF NO$ = "" THEN 10
390 GOTO 120
392 PRINT: PRINT : INPUT "ANOTHER TEST READING? (Y/N)";R$
393 IF R$ = "N" THEN 400
394 FOR I = 1 TO 12:V(I) = 0:C(I) = 0: NEXT
395 HOME: CLEAR :NO$ = ""
396 GOTO 30
400 PRINT CHR$(4) "RUN DIRECTORY,D1"
410 REM **** SYSTEM  TEST ****
420 C5 = 175: HOME: PRINT CHR$(12)
430 VTAB 1: PRINT "CONTROL        INJECTED"
440 N = 30: FOR I = 1 TO N: FOR J = 1 TO 12
450 C = PEEK (D(J))
460 IF C < C5 THEN V(J) = V(J) + 1
470 IF C > C5 THEN C(J) = C(J) + 1
480 NEXT
490 FOR J = 2 TO 12 STEP 2: VTAB J: PRINT V(J),V(J-1): NEXT
500 NEXT : PRINT CHR$(7); CHR$(7): GOTO 340
```

Figure 2. Program in basic to gather data in the PIFIR.

(AUC) relating FI versus time was obtained by the trapezoidal rule [Gibaldi and Perrier, 1975; Rowland and Tozer, 1989]. Control rats and rats injected with uric acid/mineral oil were compared by analysis of variance (ANOVA) and Dunnett's test. The AUCs of morphine administered by differents routes were compared using ANOVA. Values of $P < 0.05$ were considered statistically significant. ED_{50} values were calculated by a polynominal program fitting of two variables in iterative form taking in account the maximal effect possible in this model, 375 units of AUC. The E_{max} and T_{Emax} were obtained directly from the FI against time curves of doses that produced the highest AUC of each drug.

RESULTS

Histopathology

Intra-articular injection of uric acid in the right hind knee produced local acute inflammation. Histopathological analysis showed edema and inflammatory infiltration in synovial membranes with no effect on the joint cartilage (data not shown). Groups of polymorphonuclear leukocytes forming masses or free filaments were present in the articular cavity. Fibrin, hemorrhage, or necrosis were absent and no synovial cell proliferation was detected. The intensity of the inflammatory lesion was moderate as reflected by formation of polymorphonuclear leukocyte exudate.

Time Course of Functionality Index (FI)

The time course of FI was followed for 6.5 h measuring every 30 min (Fig. 3). FI was 100% at the start of the experiments, i.e., the time of contact (sec) between both hind limbs was similar. Control rats injected with 0.05 ml of mineral oil did not show any significant decrease ($P > 0.05$) of FI during the whole period of observation. Intra-articular injection of uric acid suspension induced a dysfunction of the injected limb that was apparent as a gradual reduction of FI with a maximal decrease at 2.5 h leveling at zero for the remainder of experiment (6.5 h). Morphine (10 mg/kg sc) administered at 2.5 h when FI approached zero produced a gradual recovery. Values reached a maximum of about 75% and then decreased slowly.

Effect of Morphine Administered by Various Routes

The FI values obtained with morphine after i.p., i.m., and p.o. administrations are depicted in Figure 4. Doses of 10 mg/kg i.m. or 31.6 mg/kg i.p. yielded results similar to those observed with 177.8 mg/kg p.o. ($P > 0.05$). FI reached a maximum of about 70% to 80% in 1 h and then decreased gradually. An oral dose of 177.8 mg/kg resulted in a relatively rapid increase of FI over a 1.5 h period, followed by a sustained effect for the remainder of experiment. These results were consistant with the pharmacokinetic profile of morphine [Jaffe and Martin, 1991].

Effect of Reference Agents on the Functionality Index

The time-action course of selected analgesic and antiinflammatory agents on the FI are shown in Figure 5. Morphine (5.6 mg/kg sc) induced a rapid increase in FI which reached a maximum of about 70% in 1 h and then decreased. Acetylsalicylic acid (316.2 mg/kg p.o.) produced a maximal functionality index of 45% in 1 h and values remained stable for 4 h. Acetaminophen (177.8 mg/kg p.o.) increased the FI by 27% in 1 h followed by a rapid decline to zero in 3 h. Indomethacin (2.4 mg/kg sc) induced a slow but steady increasing FI, reaching 70% in 4 h. For this reason the maximal effect of indomethacin could not be established within the 4 h observation period. Hydrocortisone (50 mg/kg sc) exhibited a latency period

TABLE 1. Some Pharmacological Parameters of Opiates and Nonsteroidal Antiinflammatory Drugs Determined in the PIFIR Model

Drug and dose (mg/kg)	E_{max}[a] (FI)	X ± SE	T_{Emax}[a] (h)	AUC (% . h)	X ± SE	ED_{50} in PIFIR[b] (mg/kg)	X ± SE
Morphine (17.8 sc)	96.6	12.6	1.5	310.2	25.3	7.9	1.3
Pentazocine (31.6 sc)	78.2	26.5	3.0	241.8	72.1	30.2	5.4
d-Propoxyphene (316.2 sc)	87.2	6.0	4.0	204.1	17.2	208.2	4.1
Indomethacin (3.16 sc)	74.1	10.9	3.0	227.4	30.1	2.6	1.3
ASA[c] (562.3 p.o.)	83.9	7.5	2.5	258.3	54.6	399.6	1.9
Dipyrone (1,000 sc)	87.0	5.2	2.5	268.9	15.34	517.5	1.2

[a]Variables measured of curves of time course.
[b]Parameter obtained taking as E_{max} the general maximal effect in this model in the present form (375 area units).
[c]Acetylsalicylic acid.

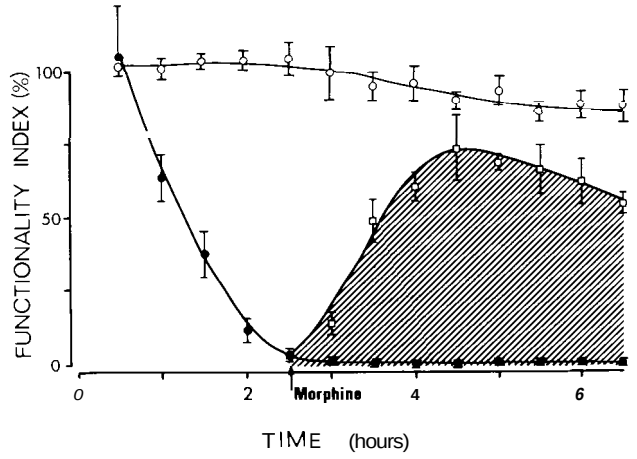

Figure 3. Time course of FI. (○), Control rats (intra-articular injection of 0.05 ml of mineral oil in the right hind knee). (●) Uric acid rats (intra-articular uric acid in the right hind knee). (□), Uric acid-morphine rats (10 mg/kg of morphine sc 2.5 h after uric acid injection). Data are presented as mean ± SE of six animals. $P > 0.05$ between control rats and temporal course of rats injected with mineral oil.

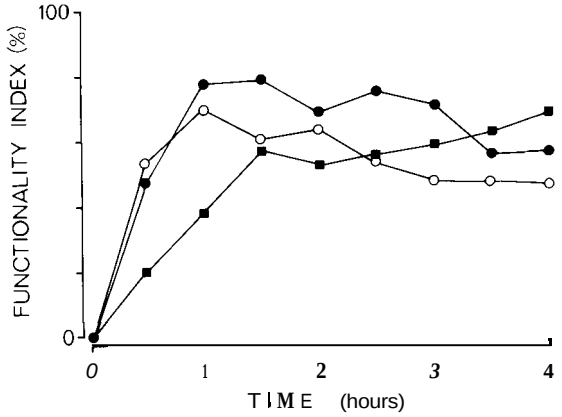

Figure 4. Time course of FI in rats injected with uric acid treated by morphine 2.5 h after uric acid injection. (●) 10 mg/kg i.m.; (○) 31.6 mg/kg i.p.; (■) 177.8 mg/kg p.o. Symbols represent the mean ± SE for at least eight animals. $P > 0.05$ between these groups.

of 1.5 h, prior to any effect being seen, and reached a maximum of 40% in 3–4 h. The doses used were selected from dose-response curves obtained previously.

The observations with different compounds suggest that the temporal patterns of drug action may reflect the pharmacological properties characteristic to a class of compounds. For example analgesic agents had a fast onset while antiinflammatory compounds were slower acting.

Expression and Analysis of Experimental Data

The results with morphine, pentazocine, d-propoxyphene, indomethacin, and dipyrone were administered sc and acetylsalicylic acid given orally are presented in Figure 6. In all cases AUC increased in a dose-dependent manner. Indomethacin and morphine appeared to be the most potent of the assayed compounds but indomethacin showed a limited efficacy with a maximum at 227 area units. Pentazocine yielded a parallel curve but shifted to the right compared to that of morphine. A lower potency as well as a limited efficacy were observed with d-propoxyphene, which reached its maximum at about 200 area units. Acetylsalicylic acid p.o. and dipyrone sc yielded similar dose-response curves. They were considerably less potent than indomethacin but they exhibited a higher efficacy. A summary of parameters as such E_{max}, T_{Emax}, AUC, and the ED_{50} of the drugs from the data shown in Figure 6 are documented in Table 1.

DISCUSSION

The PIFIR method appears to be suitable for the assay of analgesic drugs but is not suitable to characterize agents such as indomethacin because of their delayed onset and long duration of action. The deter-

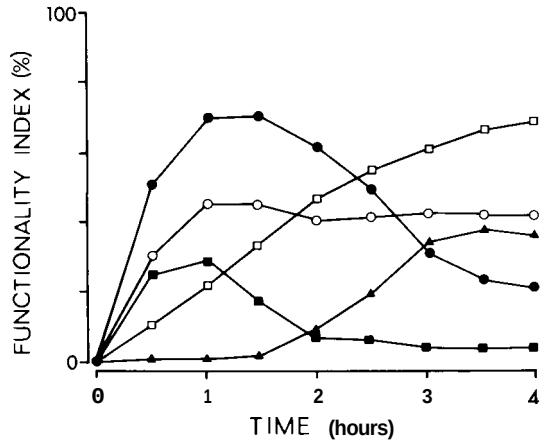

Figure 5. Time course of FI in rats injected with uric acid treated by analgesic and antiinflammatory agents. (●) Morphine 5.6 mg/kg sc; (○) acetylsalicylic acid 316.2 mg/kg p.o.; (■) acetaminophen 177.8 mg/kg p.o.; (□) indomethacin 2.4 mg/kg sc, and (▲) hydrocortisone sc 50 mg/kg. Symbols represent the mean ± SE for at least six animals.

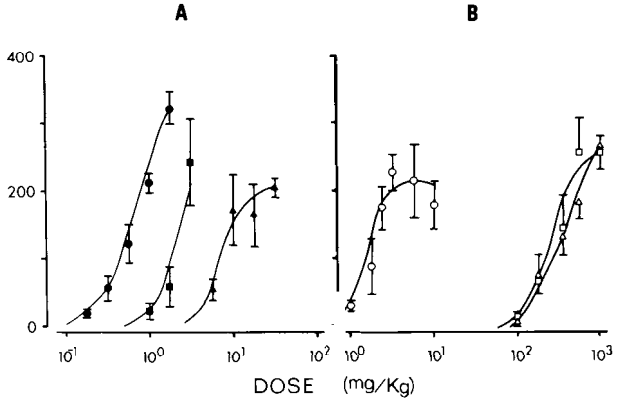

Figure 6. Dose-response curves of several analgesic agents determined in the PIFIR. The response is expressed as AUC (vertical axis) estimated over a 4 h observation period. Left panel (A): (●) morphine sc, (■) pentazocine sc, and (A) d-propoxyphene sc. Right panel (B): (○) indomethacin sc, (△) dipyrone sc, and (□) acetylsalicylic acid p.o. Symbols correspond to the mean ± SE for at least six determinations.

mination of the time course of study would accommodate such compounds.

Histopathological analysis showed that uric acid induced acute inflammatory response comparable to that seen with intra-articular injection of sodium urate crystals [Faires and McCarty, 1962; Van Arman et al., 1970]. After uric acid injection, there was a motor dysfunction of the injected extremity, i.e., the rats avoided the use of the injured hind limb when forced to walk. The onset of dysfunction was gradual with a maximal (almost 100%) effect in 2.5 h. These features were similar to those reported with sodium urate-induced effects in dogs [Van Arman et al., 1970] and cats [Okuda et al., 1984].

It has been reported that the leg dysfunction is mainly produced by the inflammatory process because it was reversible by antiinflammatory agents [Van Arman et al., 1970]. However, Okuda et al. [1984] have provided evidence using morphine that animals avoid using the traumatized extremity chiefly because of a manifestation of pain. In our experiments we observed that drugs without antiinflammatory actions, such as morphine, exhibited high potency, and efficacy. Conversely, pure antiinflammatory agents, such as hydrocortisone, showed a different activity and the onset of the effect was very slow (indomethacin was active but was observed over too short a period). These results are in agreement with those of Okuda et al. [1984] and suggest that leg dysfunction is primarily due to pain caused by acute inflammation.

Uric acid injection produced a persistent dysfunction of the injured limb which lasted at least for 4 h (Fig. 3). Therefore, the procedure can be considered as a model of tonic pain and thus may simulate clinical pain in temporal parameters.

The use of the FI has the advantage that the noninjured limb serves as control. Drugs having effects on the motor activity of the animals should also affect the functionality of the control limb; therefore the use of the FI enables to minimize this type of interference. The FI is determined when rats are forced to walk. This is a natural behavior of the animals and thus lengthy training sessions are not necessary. Rats get used to the rotating apparatus in less than 2 h. The use of small animals as experimental subjects presents the advantage that significant amounts of drug are not required.

Many pharmacological assays, like those for drugs affecting the cardiovascular, respiratory, gastrointestinal, or endocrine systems [Holford and Sheiner, 1981], permit the evaluation of efficacy as a function of time. In the area of analgesia, traditional procedures, such as the "tail flick" or the "tail clip," evaluate behavioral responses like squeaking or biting after application of an acute stimulus. Therefore, after several stimuli there is a conditioned learning effect interfering with the response. In the PIFIR method, the noxious stimulus is applied only once and produces a constant pain level.

In addition to variations in potency, there were important differences in the time courses of the antinociceptive effect induced by the agents studies. Morphine (ip) had a fast onset which diminished after reaching a maximum. Acetylsalicylic acid (po) resulted in a more persistent effect, although the doses

needed were quite high. Indomethacin (sc) induced an effect of considerable magnitude but of slow onset.

From the data in Figure 5, it is misleading to consider the analgesic efficacy at a fixed arbitrary time (for example 2 h after drug administration). The construction of FI versus time curves allows the direct determination of the maximal effect as well of the time to reach the maximum (Table 1).

The AUC is a parameter that considers the overall kinetic profile, including the maximum as well as the duration of the effect. For this, some drugs can have the E_{max} similar (i.e., d-propoxyphene and dipyrone, Table 1) but the AUC or integrated analgesic effect could be different. Therefore, the parameter AUC was chosen for the construction of dose-response curves.

The ED_{50} values were calculated taking in account the maximal effect possible in this model (375 area units) because in this way it is possible have a parameter of comparison for all the drugs without an arbitrary E_{max}, i.e., as in the case of morphine.

In conclusion, the method here presented has the following advantages: i) it allows the use of rodents for studies of pain, ii) it permits determinants of the time course of the analgesic effect in the same animal, iii) it does not require any long training period. The PIFIR method is not presently suitable to characterize agents such as indomethacin because of their delayed onset and long duration of action, but it could be modified the necessary time for evaluating.

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