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Review

A Critical Analysis of the Experimental Evaluation of Nociceptive Reactions in Animals[1]

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Because of the complexities of the central nervous system compounded with the ambiguities of interpreting the sensation of and reaction to "pain" in animals, available techniques for experimental evaluation of nociception to various types of stimuli are crude. Simple methods are often associated with artifacts that affect both the qualitative and the quantitative outcome of the test. It is therefore mandatory to control every detail of the experimental protocol. Further, the acuity expected from the experimenter to observe the animals during the test cannot be overemphasized. Additionally the test procedure should not inflict excessive suffering to the animals. It is the purpose of this review to highlight some of the "safeguard" measures to be adopted in order to avoid false-positive and false-negative results and erroneous interpretations and conclusions.

KEY WORDS: nociception; opioids; *in vivo* methods; analgesic testing; naloxone; learning.

INTRODUCTION

The objective of this review is to focus on some of the important aspects of *in vivo* methods currently used in experimental animals to study pain. While using the animals to study pain, the investigator should safeguard their rights in accordance with the ethical values and prevailing laws. To study pain, it is unfortunately necessary to inflict a certain amount of pain. However, the degree of pain inflicted should not cause intense emotional reactions, indicative of prolonged agony. The experimental protocol should minimize the suffering of the animals and ensure that they are humanely treated.

THE MEANING OF "PAIN" IN HUMANS VERSUS EXPERIMENTAL ANIMALS

According to the International Association for the Study of Pain, pain is "an unpleasent sensory and emotional experience associated with actual or potential tissue damage." This definition distinguishes the pain under examination from the "pain" (grief) of losing a loved one, the pain (disappointment) of unfulfilled expectation, or the pain (exasperation) of attending an interminable meeting. It also recognizes that pain can and frequently does arise in the absence of noxious stimuli. Pain is not a simple sensation caused by a specific stimulus but rather a complex reaction and experience with a multidimensional quality; it obviously varies widely among individuals and even in the same individual at different times. Painful stimuli also influence affective reactions that interact with the sensory components of pain. With these considerations, pain or nociception is viewed as a complex experience, comprising a sensory component referring to the qualitative sensory experience elicited by the stimulus and a reactive component that refers to the accompanying affective and emotional response. In experimental animals, it is not possible to obtain verbal reports. Also, it is not clear whether animals perceive pain in the same fashion as human subjects, and the pathways transmitting nociceptive messages, brain transmitters, and their receptors in animals are similar to those in humans. With reference to animal experimentation, it would be helpful to define pain in an operational sense based on stimulus conditions and observable responses. The term "painful" is usually used synonymously with the term "nociceptive" or "noxious," which means destructive or tissue damaging. Alternatively, the term "aversive" is frequently applied to stimuli that elicit behavioral responses to avoid stimulus conditions. "Nociception" is a better term to use in animals than "pain."

CONSIDERATIONS REGARDING NOCICEPTIVE STIMULI

A nociceptive stimulus must be carefully selected since there is a variety of stimuli, differing in applicability and limitations. None of the current techniques meets all the requirements of an ideal nociceptive stimulus. The parameters for the nociceptive stimulus must be quantifiable and controlled with precision in order to minimize variability of ex-

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perimental results due to fluctuations in stimulus parameters; the stimulus used should simulate as far as possible natural conditions (1); the stimulus must be easily and frequently repeatable. This aspect is problematic because tissue damage alters the response by either sensitization or reduced sensitivity; further, repeated presentations of nociceptive stimuli lead to anticipatory avoidance learning, which interferes with the testing process (2). Therefore the right choice of a stimulus may frequently depend upon the exact nature of the experimental condition and the types of responses. Since different classes of analgesics vary in their mechanisms of pain relief, it is recommended not to rely on any one form of nociceptive test during the determination of analgesic efficacies.

RESPONSES TO NOCICEPTIVE STIMULI IN EXPERIMENTAL ANIMALS

Whereas humans can express and distinguish a wide variety of painful sensation, animals can display only autonomic and/or somatomotor disturbances. Somatomotor responses are most commonly employed in the experimental analyses of pain. Some of the somatomotor responses are the tail flick and writhing in the mouse, vocalization in the rat, etc. The tail-flick response can be elicited in chordotomized animals and therefore involves polysynaptic reflexes; however, in normal circumstances it involves long supraspinal pathways (3). Nociceptive responses such as jumping in the hot-plate test, writhing to a chemical stimulus, and vocalization to an electrical stimulus require a high degree of sensory motor coordination. Repeated presentation of the nociceptive stimuli modifies the responses following local alterations (which might or might not be accompanied by noticeable tissue injury), recruitment, facilitation, inhibition, and/or conditioning. Jacob (4) has shown that with single exposure, either a high temperature of the hot plate or a long period of contact of the paws is needed to elicit a jump response; with repeated exposures, jump occurs at a much lower temperature of the hot plate and replaces the licking reactions ("occlusion"). These phenomena do not occur if the container is low and covered, as the animal learns that it is helpless ("learned helplessness"), and therefore they depend on the awareness of the environment; once acquired, these behavioral alterations resist extinction at least for 24 hr (memory). These phenomena can be partly reversed by electroshock or by extinction procedure by putting the mice repeatedly on the same apparatus, except not heating the plate. Conditioning might occur even in simple tests involving spinal reflexes when the animals are repeatedly tested at short intervals.

QUANTITATIVE DETERMINATION OF THE ACTIVITY OF ANALGESICS

Quantification of the activity of drugs differs from one test to another. Depending on whether or not the nociceptive stimulus is kept constant, the methods can be divided into two groups. In the first instance the activity of the drugs is related to the disappearance of responses. Graded responses can be used either by scoring (the intraarterial bradykinin test) or by measurable features (latencies in the hot-plate test and the number of abdominal contractions in the writhing test). In the second case the activity of the drug is related to the stimulus variations to elicit the responses. These variations might be related to the duration or the intensity of the stimulus or both. When keeping the intensity constant, one measures the reaction time. However, the major disadvantage here is to adopt a "cutoff time" which alters the statistical distribution and the calculations. If the duration of the stimulus is kept constant, one then measures the thresholds. In reality, the duration is rarely kept constant, since it increases as the threshold is measured. When it is really kept constant, repetition of the stimuli influences the results and interferes with the objective. When the time course of the effect is desired, it is necessary to use separate groups of animals instead of repeated exposure of the same animals at different time intervals to avoid the interference of learning.

SOME COMMONLY USED NOCICEPTIVE TESTS

A great variety of nociceptive tests is currently used, differing from each other by the nature of the stimuli, parameters, sites of application, nature of responses, quantitation, and apparatus. Objectively, depending upon the nature of the stimulus, they can be classified into chemical, electrical, mechanical, and thermal methods.

Chemically Induced Nociception

A variety of chemical agents has been employed to produce pain. The intraperitoneal administration of a noxious chemical substance to mice and rats produced peritoneal irritation, which elicits a writhing response. This response is unlearned and reflexive in nature. Each episode of writhing is characterized by internal rotation of the feet, sucking in of the belly, elongation of the body, arching of the back, rolling on one side and remaining still, or turning around and circling the cage (5). Many chemical irritants have been used, which include acetic acid (6,7), acetylcholine (8), alloxan (9), bradykinin (10), hydrochloric acid (11), hypertonic saline (12), lipoxidase (13), oxytocin (14), phenylquinone (15,16), and serotonin (17). Acetic acid and phenylbenzoquinone are the two most commonly used irritants. The use of phenylbenzoquinone is associated with problems of solubility, photosensitivity, and autooxidation.

Writhing can be abolished by evisceration, intraperitoneal application of procaine, spinal transection, and ablation of the cerebellum. Midbrain decerebration eliminates arching of the back and decortication does not affect the writhing response at all (5). Writhing is also prevented by electrical stimulation of the periaqueductal gray (12). One of the major drawbacks of this test is the great variation in individual sensitivity, when the number of writhings is considered (15). Atropine, adiphenine, and dicyclomine are inactive in acetic acid-induced writhing (16), indicating that intestinal spasm is not involved in the production of writhing (18). Analgesic activity is present, if the latency to the first writhe is prolonged or the frequency of writhing is reduced. The writhing test has the advantage of simplicity and sensitivity to all clinically useful analgesics (19). Both the agonists and the agonist antagonists are readily detected in this assay (20). On the contrary, injection of the opioid antagonist naloxone increases the frequency of writhing in mice

and rats (6,21). Morphinomimetic actions of naloxone tend to predominate in the writhing test, when high doses of naloxone are employed (6,7). The major disadvantage of the writhing test, however, is its lack of specificity, generating false positives to nonanalgesics (22) and other variables (23). An auxiliary test to measure motor coordination can be utilized to keep false positives to a minimum (19). Writhing induced by the intraperitoneal injection of bradykinin can be considered as a model for visceral pain (24).

Intraarterial Bradykinin Test

Intraarterial injection of bradykinin evokes vocalization and indictions of pain in experimental animals (25–27). Intracarotid administration of bradykinin (0.5 to 0.1 μg/kg) in the rat (28) results in dextrorotatory movements of the head, flexion of the respective forelimb, and an occasional vocal response; repeated administration of bradykinin at regular interval does not lead to tachyphylaxis. Once the sensitivity of an individual rat has been established, a dose of test analgesic is administered and its effects on the bradykinin responses are scored. In general, compounds that effectively inhibit writhing are also active in the intraarterial bradykinin test, but the latter is more specific (29) and is a sensitive indicator of the analgesic effect of agonist antagonists than the tail flick test (30). Interestingly, naloxone produced hyperalgesia in a group of rats in which flexor reflexes displayed tolerance to intraarterial bradykinin (noxious adaptable group) (31). This effect of naloxone was absent in those rats which gave a consistent response to bradykinin (noxious nonadaptable group). The effect of bradykinin is not strictly limited to nociceptors and other receptors innervated by fast-conducting fibers are concomitantly excited (32,33). Despite these data, bradykinin is still considered as an endogenous transmitter in peripheral mechanisms of pain (34).

Ethylenediamine Tetraacetic Acid-Induced Nociception

Intradermal injection of ethylenediamine tetraacetic acid in the guinea pig evokes vocalization, biting, scratching at the site of injection, and escape behavior (35). Both opioid agonists and mixed agonist antagonists are active; the nonanalgesic drugs are inactive (35). This test has been suitably adopted in mice (36). However, the effects of varying doses of naloxone have not been reported so far in both species.

Formalin Test

Subcutaneous injection of formalin in the dorsal surface of the forepaw of the rat elicits a series of responses such as elevation and licking of the paw, which are scored from 0 to 3 (37): 0 for full weight placed on the paw, 1 when the injected paw rests lightly on the floor and bears no weight, 2 when the injected paw is selectively elevated and in contact with no other surfaces, and 3 when the paw is licked or bitten. Morphine-type opioid analgesics are effective in this test (38). Naloxone did not produce hyperalgesia in the formalin test because of an already attained maximal response (too high a concentration of formalin).

Electrical Stimulation Methods

Electrical Stimulation of the Tail

Electrical stimulation of the tail through intracutaneous needles in animals (39,40) produces consistent responses. Three types of pain thresholds are determined following the electric shock applied to the tail (41), corresponding to three different levels of integration of pain within the central nervous system: (a) a low-intensity stimulation produces a motor response, tail withdrawal (which is a low-grade spinal reflex); (b) a higher voltage induces a simple vocalization involving the lower brain stem; and (c) a stimulation using a higher voltage produces a brief vocalization after the stimulation is terminated (vocalization after discharge), representing the affective component of the pain response involving hypothalamus and rhinencephalon. During the test the animal is restrained with its tail free. The voltage is progressively increased stepwise until the three thresholds are successively determined. The standard experimental procedure consists of testing the animals three times at 15-min intervals during 3 consecutive days, after which they become adapted to testing sessions, by the absence of stressful symptoms such as defecation, teeth chattering, and excessive agitation; they show no untoward reaction during the insertion of the needles. Thus the threshold values obtained on the third day are the baseline values. High doses of morphine were necessary to antagonize vocalization, whereas low doses of morphine could block effectively the vocalization after discharge (9). Thus it is possible to differentiate between responses that are integrated at various levels in the pathways of nociception. Carrol and Lim (9) showed that brain transection between thalamus and midbrain blocked the vocalization after discharge, while higher ablations were without effect. Transections caudal to the medulla blocked vocalization during stimulation as well as vocalization after discharge. These findings reveal that morphine-induced analgesia results from the initial blockade of nociceptive thalamic afferents, followed by blockade of brain-stem and spinal neurons. This method is sensitive to opioid agonists, antagonists, and antiinflammatory agents. The opioid antagonist naloxone has been shown to decrease the threshold for vocalization and vocalization after discharge (42).

Flinch-Jump Test

In the flinch-jump technique, a constant-current shock is applied to the grid floor of the cage and the behavior of the animal is noted. The current level is either increased or decreased after each presentation and the order of shock intensity presentation is determined by the ascending and descending series. As the shock intensity is increased from zero, the first behavior is flinch; at higher levels of shock intensity, the animal escapes to avoid the shock. This test retains the advantages of good sensitivity to opioid and nonopioid analgesics (43). Opioid agonists and mixed agonist antagonists elevate the jump threshold without affecting the flinch threshold (44), and a ceiling effect for raising the jump threshold is observed for nalorphine and pentazocine (30). This technique allows for free movement of the animal and requires no training, and multiple levels of nociceptive stim-

ulation can be studied in a session using a single animal. Repeated testing at hourly or daily intervals does not alter significantly the thresholds of any of the response categories. Each of these response types involves different central nervous system structures mediating rapid responses to foot shock (43). Lesions of the dorsomedial tegmentum, septal nucleus, or median forebrain bundle lower the shock threshold for the jump response (43).

Trigeminal Nerve Stimulation

In the rat, direct electrical stimulation of the ophthalmic division of the trigeminal nerve evokes a vocal response (45). More refined electrophysiological approaches to assess neuronal activity in the trigeminal nucleus can also be performed.

Shock Titration Technique

In the shock titration paradigm (46,47), the experimental subject has operant control over the intensity of a cutaneous electrical shock, with the shock intensity increasing in a stepwise manner unless suppressed by a bar press. The test animal therefore determines the tolerated level of shock. When stabilization of the shock threshold is achieved with daily training sessions for 7–10 days, the monkeys are then adopted to once-a-week sessions and are tested thereafter at weekly intervals (48). The behavioral measures frequently used are the response rate or the amount of time spent at each stimulus intensity. As this technique can provide a continuous record of escape threshold, it is ideal for studying the time course of analgesic drugs (49). Some of the disadvantages of this method are that (a) variations in the size of the increment or decrement produced by the behavioral response affect the titration threshold, (b) inconsistencies are observed in this technique in the effects of some drugs, and (c) the aversive thresholds in the titration methods might really reflect avoidance thresholds and that the animals regulate shock intensity to levels below those actually perceived as nociceptive (49). The electrical stimulation of the foot seems to have a lesser sensitivity than that of the gausserian ganglion. This method can detect levels of morphine (0.1 mg/kg) (30). Cyclazocine (50), but not nalorphine, elevates the shock threshold and requires 100 times more naloxone for reversal than that of morphine (47). Nonsteroidal antiinflammatory analgesics are inactive in this test (48). Side effects of the test drugs (sedation, motor deficits, mood changes such as euphoria and dysphoria) might influence responding in addition to any perceptual effects. In rhesus monkeys, morphine produces side effects on sexual behavior (51) and food-reinforced responding (52) in the same dosage range used to produce analgesia (53,54). One cannot therefore, with confidence, describe a reduction in the response to nociceptive stimuli as analgesia, when responses to other types of stimuli are reduced.

Tooth-Pulp Stimulation

Electrical stimulation of the tooth pulp has been used in antinociceptive tests (55–58) and is a useful model for studying facial pain (49). Considerable controversy exists, however, over whether receptors in the tooth pulp have an exclusive role in mediating nociception. The findings that the innervation of the tooth pulp consists exclusively of A-δ and C-fibers indicate that they conduct nociceptive information. Nonnociceptive sensations might be explained by the spread of electrical stimulus outside the pulp to gingival and/or periodontal tissues, innervated by fast-conducting fibers, supplying to mechanoreceptors (59). The risk of current spread is highest with monopolar stimulation, using an "indifferent" electrode in contact with the skin in the hand or earlobe, etc.; under these conditions, the receptors other than those in the tooth might be excited (60). Moisture can cause current to be shunted along the surface of the tooth to gingival tissues and result in reduced current flow through the pulp, with the resultant stimulation of nonpulp receptors (49). These problems might partially be solved by using bipolar electrodes. An appropriate stimulator can be used to stimulate the pulp. Tetanic stimulation of the tooth pulp produces a nociceptive reaction in an awake cat which is graded according to the stimulus intensity (61). With increasing voltages the sequences of responses elicited are jaw opening, hyperextension of the head, jaw opening and rotation of the head, and scratching of the tooth. The suppression of the jaw-opening reflex evoked by tooth pulp stimulation is considered to be an accurate index of analgesia and is sensitive to opioid agonists, mixed agonist antagonists, and centrally acting nonopioid analgesics (62). This test has been adopted in freely moving rats. The dose–response curve slope for aspirin is shallower than that of the opioid analgesics; naloxone produces no significant changes in threshold responses. The opioid-induced increase in the response threshold in this test is not directly related to the depression of the central nervous system (63).

Mechanical Stimulation

Selective stimulation of the mechanoceptors can be achieved by the application of high pressure. When applying high pressure, it is not possible to avoid stimulating low-threshold mechanoceptors. The disadvantage of the method is that the stimuli might produce receptor damage and therefore repeated application may not elicit reproducible results. Various adaptations of the Von Frey technique (64), using hair or nylon strings of different diameters and lengths, are the most common methods of quantitative expression of pressure. The threshold for evoking nociception is usually above 40 g/mm^2.

Tail-Clip Test

The tail-clip test was introduced by Haffner (65). Bianchi and Franceschini (66) extended this method by using a "constant" stimulus by means of an artery clip, the arms being enclosed in a rubber tube. The artery clip is applied to the base of the mouse's tail for 30 sec. The response consists of attempts to dislodge the clip by biting it, thus the oriented biting involves higher centers in the central nervous system. Opioid analgesics cause the mice to be indifferent to the clip. Clips can also be applied to other parts of the body such as ears, paws, and toes. The effects of naloxone and other opioid antagonists in this test are not yet reported.

Tail Compression Test

In the caudal compression test (67), the stimulus is applied to the tail of the rat. Threshold pressures are measured with two syringes connected by means of a flexible tubing filled with a fluid and the pressure in the side arm is measured in the manometer. The rat responds to pressure, first by struggling, then by vocalization; the latter is regarded as the most specific central indicator of nociception in animals (68). The tip of the tail is best for obtaining a prompt response.

Inflammatory Pain

Hyperesthesia can be induced in the rat hind paw by subplantar injection of an aqueous suspension of brewer's yeast or carrageenan. The sensitivity to pressure is measured an hour later (69). A vocalization response to the applied pressure can be quantitated. Opioid analgesics raise the threshold in the noninflamed as well as the inflamed feet; the mixed agonist antagonists raise the threshold only in the inflamed feet (68). The antiinflammatory analgesics of the indomethacin type show analgesic activity only in the inflamed foot. Interestingly, naloxone administration in the inflamed paw antagonizes the inflammatory pain produced by intraplantar administration of carrageenan in rats (70) and the analgesic action of naloxone in this test might be due to its action on cutaneous opioid receptors and/or local production of morphinomimetic metabolites by dealkylation of naloxone (71).

Toe-Squeezing Method

In the multiple toe-pinching test in the guinea pig, opioid and some nonopioid analgesics are effective (72). Manual toe squeezers are also used to evoke a flexor reflex in chronic spinal dog (30). In this test, there is little difference in the potency of morphine or cyclazocine. However, nalorphine produces greater depression of the flexor reflex, when the weakest stimulus is employed (30). The mechanism of depression of the flexor reflex by cyclazocine and pentazocine in the chronic spinal dog appears to be quite different from the spinal cord effects of these drugs in the rat and therefore it has been suggested that the rat spinal cord is considered to be devoid of kappa receptors (30).

Thermal Stimulation

A thermal stimulus has the advantage that it is easy to control and does not stimulate the mechanoceptors. Conducted heat stimuli could be easily applied to a moving subject or to immobilized animals. Two reactions, the skin twitch and the escape or withdrawal, occur at average skin temperatures of 45 to 46 and 51 to 52°C, respectively, in experimental animals. Changes in the skin temperature by drugs should be carefully monitored to avoid alteration of pain thresholds and misleading results (73).

Tail-Flick Test

The tail-flick procedure of D'Amour and Smith (74–76) has become a standard screening procedure for analgesics in both mice and rats. The rodent is confined with the help of a cloth towel or a plastic holder so that it is relatively immobile and is kept calm. A radiant heat from an electric source is focussed on the marked end of the tail. The intensity of the heat stimulus is varied so that the tail flick-latency can be carefully selected. The tail-flick latency can be accurately and automatically recorded without tissue injury. A suitable cutoff time can be used. Generally two predrug control reaction times are determined 15–30 min apart. Drug is then administered and the tail-flick latency measured at 15- to 30-min intervals. An ED_{50} can be calculated from the number of animals that do not respond within the cutoff time (10–20 sec, depending on the protocol). At low intensities, most agonist antagonists are not detected (77). Naloxone does not affect the reaction time in mice, whether the control tail-flick latencies are short or long (78,79). But Berntson and Walker (80) reported a moderate reduction in tail-flick latency in rats by naloxone, which could not be reproduced (79). The strength of the stimulus does not appear to play a significant role and may not be the sole reason for the lack of effectiveness of mixed agonist antagonists in this test (30). The animals used should be young so as to minimize the heat insulating effect of keratinization in the tail. The color of the skin in the tail area should be consistent to standardize the heat absorption (76). The locus of tail stimulation in the tail-flick assay is also important, as the distal tail section is more sensitive to the analgesic effects of morphine than the proximal sections (81). Tail vein injections should be avoided in the tail-flick procedure. It is also necessary to control the skin temperature of the tail, when using radiant heat (73,82). The restraint stress in this test might itself activate endogenous opioid and/or nonopioid pain inhibitory systems which interfere with the test (83). The tail-flick response does not involve a high degree of sensory–motor coordination, as the response varies little between intact and spinal animals (3). Although being a low-grade spinal reflex, the tail-flick response is normally under the physiologic control of higher centers. In rats transected at high thoracic levels, the tail-flick reflex is more exaggerated than usual, probably as a result of abolition of descending inhibition from higher centers (82), and partly due to peripheral vasodilatation following spinal transection. Spinal mice exposed to radiant heat stimuli still flick their tails (84). Electrical stimulation of the paraventricular gray areas of the brain causes an increase in the tail-flick latency (85). Intraventricular injection of morphine causes an increase in tail-flick latency, supporting the hypothesis that supraspinal mechanisms are also involved in the actions of opioids. Repeated testing at short intervals might also affect the tail-flick latency under treated conditions. In these circumstances it is very difficult to distinguish whether the observed modification is due to the measure of repeated testing alone or due to the effect of the drug treatment. Two other important factors are, the cutoff time and the effect of the drug treatment on body temperature.

Tail Immersion Test

The rodent tail withdrawal reflex can be elicited by immersion of the tail in hot water from 45 to 65°C and is sensitive to opioid analgesics (86). At low stimulus intensities (45°C), some mixed agonist antagonists can be detected

(87,88). The effect of naloxone under different stimulus intensities in this test is not yet reported. The nonopioid analgesics such as cyclooxygenase inhibitors are found to be generally effective at low temperatures (45°C) (89). The end point might be a major spinal reflex. Young animals should be used and injections in the tail should also be avoided (76).

Hot-Plate Technique

In the hot-plate technique, a thermonociceptive stimulus is applied to the paws of the animal, which can move freely inside the container (90). Several modifications of this test have been described. In the commonly used test of Eddy and Leimbach (91), the temperature of the hot plate is kept constant. The responses of the animal can be easily observed through the glass cylinder open at both ends. The animal is gently introduced onto the hot plate. The responses are agitation, rapid withdrawal of the paws, "dancing" (not used in analgesic measurements because of a low proportion of mice exhibiting this response), licking of the forepaws and/or hind paws, and jumping (simple jump, adjusted jump so as to sit on the top edge of the cylinder, or jumping off of the hot plate). The analgesic activity of a test drug cannot be tested using a closed cylinder because of the interference of learned helplessness. The most commonly used responses are licking and jumping and the experimental animal is the mouse or rat. A complete analysis of the action of any substance can be made after measuring the various behavioral reactions on the hot plate. For example, the effects of morphine on the licking and jumping responses are not identical, because the licking response on the hot plate is less sensitive to morphine than the jumping reaction. These differences indicate that opioidergic pathways are predominantly involved in a complex response such as jumping, and to a lesser degree in licking. Some drugs affect the licking and jumping in different ways. For example, atropine-like drugs inhibit selectively the effects of morphine on the jump through a central mechanism (92,93) and *d*-lysergic acid diethylamide preferentially affects the licking, and other hallucinogens affect both responses at the same time (94). Low doses of naloxone produce hyperalgesia as measured by decrease in licking and jumping latencies in mice and rats (95–97) and this has been confirmed (98,99). In order to observe the hyperalgesic effect of naloxone, test naive animals and use a limited number of preexposures; when the number of preexposures decreased the jump latencies to very low values, naloxone increased the jump latencies (2,100). Many investigators determine the baseline nociceptive threshold by repeated testing of the animals in order to stabilize them without realizing that concomitant learning occurs, which interferes with this method. The licking reactions are also modified by naloxone, provided the temperature of the hot plate does not exceed 50°C. At a hot-plate temperature of 55°C, the licking latency has already attained maximum velocity as represented by the lowest values and therefore could not be further decreased by naloxone. The jump response is a very complex one, involving awareness to the environment even on the first exposure, because facilitation occurs on the second exposure. This type of learning could be inhibited, if the environmental conditions relating to the first exposure are inadequate (4). Nevertheless, by reducing the temperature of the hot plate to 49.5°C, an analgesic action of pentazocine, nalorphine, and levallorphan could be detected in the rat (101). This test is probably less sensitive than the tail flick. However, the importance of this test is that it relies on a more complex and integrated response of the experimental animal, thus reflecting processes occurring at a higher level of the central nervous system, and is most commonly used to assess analgesic effects of opioids and nonopioids (102–104), hyperalgesia produced by opioid antagonists, amine antagonists (105–107), and other drugs. The analysis of genetic variations might also be helpful in the elucidation of various aspects of the regulation of nociception (108). For example, CXBK-strain mice are poor in cerebral opioid receptors and/or their ligands and they respond rapidly in the hot-plate test at 55°C (109). On the other hand, CXBH-strain mice appear to be rich in cerebral opioid receptors and/or their ligands and therefore they are slow responders to the thermonociceptive stimuli (109). CXBK- and CXBH-strain mice appeared to be deficient in kappa receptors and/or their ligand levels relevant to thermonociception (110). At present mu, kappa, and delta types of opioid receptors are all implicated in the regulation of thermonociception.

Two other tests using heat are the ultrasound procedure (111) and laser-induced nociception (112).

MODELS FOR CHRONIC PAIN

Ethical problems have hampered the development of chronic pain models.

Dorsal Root Rhizotomy

Rats with dorsal root lesions at the cervicothoracic spinal cord level scratch vigorously and denude the skin; gradually this process proceeds to self-multilation of varying degrees in the deafferented limb (113), whereas small or no changes in the behavior appear after unilateral lesion of lumbar roots or the sciatic nerve (114). The abnormal sensation might be due to a sensitization of central synapses, after the partial or total deafferentation, thereby provoking hyperactivation of neurons (114) or the development of "quasi-epileptic foci" (115,116). The deafferentation hypersensitivity in the rat after dorsal root rhizotomy is reported to be a possible animal model for chronic pain (113). This model favors a comparison between the behavior of these animals and the sensations described by human subjects after lesions in the different levels of nervous systems. However, elaborate experiments are necessary to evaluate the possible value of this model.

Adjuvant-Induced Arthritis

Intradermal injection of *Mycobacterium* with Freund's adjuvant into the tail of rats has been used as a model for polyarthritis (117) and is sensitive to opioids and nonsteroidal antiinflammatory drugs. Hypersensitivity to pain develops within a few hours and is found stable for testing at 18 to 24 hr.

Model for Paroxysmal Pain

Local injection of strychnine, alumina gel, or tetanus toxin into certain parts of the brain in animals might be a

model for paroxysmal pain. Injection of these materials into trigeminal nucleus caudalis produces behavior in the animals similar to that in patients with trigeminal neuralgia (24). Application of such agents in the dorsal horn of rats is said to produce "pain syndrome of the spinal origin." Such experiments could not be used in awake animals in such a way to meet the ethical criteria of experimentation.

CONCLUSION

During the evaluation of analgesics, motor impairment can interfere with real antinociceptive activity. For example, prolongation of jumping latencies in the hot-plate test following high doses of 5-methoxy-*N*,*N*-dimethyltryptamine appear concomitantly with motor disturbances and no real antinociceptive action is evident as indicated by vigorous paw shakes of these animal on the hot plate (107). Commonly, the selectivity of the analgesic effect is determined separately by observing the motor performance on the rotarod test or inclined screen test. As an alternate, analgesia could be measured in such a way that determination of the presence or absence of analgesic activity is independent of motor performance. One such technique involves the use of an analgesic drug as a discriminative stimulus in rats with chronic arthritis (111). Despite the difficult tasks of assessing pain, anxiety, and euphoria in experimental animals, the evaluation of analgesics remains relatively easy at present. Nociceptive tests for assessing the effect of opioid antagonists and antipyretic and antiinflammatory agents should be carefully carried out. Nevertheless, newer techniques are evolving, which under appropriate conditions, could predict the analgesic and/or hyperalgesic actions of these compounds. The number and nature of tests to be employed rely on the expertise of the pharmacologist. Experiments with mice and rats have definite advantages because the activities of reference drugs are well established and documented. The nature of the test should also depend on the economy in time, the number of animals used, the reliability and reproducibility of the results, and the suitability of the results for statistical analyses. One should also be aware of the limitations of drawing analogies between animals and humans in the field of pain. *In vitro* methods might also be used. In the development of potential analgesics, attention should also be focused on side effects. Increased knowledge in the development of anatomical, behavioral, biochemical, and electrophysiological analyses of different types of nociceptive stimuli and of the responses might lead to more reliable ways of relieving pain in future.

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REFERENCES

1. H. K. Beecher. *Pharmacol. Rev.* 9:59–209 (1957).
2. J. J. C. Jacob and K. Ramabadran. *Pharmacol. Ther.* 14:177–196 (1981).
3. S. Irwin, R. W. Houde, D. R. Bennet, L. C. Hendershot, and M. H. Seevers. *J. Pharmacol. Exp. Ther.* 101:132–143 (1951).
4. J. Jacob. In Z. Votava, M. Horvath, and O. Vinar (eds.), *Psychopharmacological Methods,* State Medical Publishing House, Prague, 1963, pp. 70–79.
5. K. S. Lim, and F. Guzman. In A. Soulairac, J. Cahn, and J. Charpentier (eds.), *Pain,* Academic Press, London, 1968, pp. 119–152.
6. J. J. C. Jacob, and K. Ramabadran. *Life Sci.* 24:1959–1970 (1979).
7. J. J. C. Jacob, and K. Ramabadran. In N. E. Williams and H. Wilson (eds.), *Pain and Its Management, Int. Encyl. Pharmacol. Ther. Sect. 112,* Pergamon Press, Oxford, 1983, pp. 13–32.
8. J. J. Loux, S. Smith, and H. Salem. *Arzneim. Forsch.* 28:1644–1647 (1978).
9. M. N. Carrol, and R. K. S. Lim. *Arch. Int. Pharmacodyn.* 125:383–403 (1960).
10. R. B. P. Burns, N. J. Alioto, and K. E. A. Hurley. *Arch. Int. Pharmacodyn.* 175:47–55 (1968).
11. E. T. Eckhardt, F. Cheplovitz, M. Lopo, and W. M. Grovier. *Proc. Soc. Exp. Biol. Med.* 98:186–188 (1958).
12. G. J. Giesler and J. Liebeskind. *Pain* 2:43–48 (1976).
13. J. Cheymol, M. Freyss, and M. Beguin. *C.R. Soc. Biol. (Paris)* 98:521–524 (1963).
14. W. J. Murray and J. W. Miller. *J. Pharmacol. Exp. Ther.* 128:372–379 (1960).
15. L. C. Hendershot, and J. Forsaith. *J. Pharmacol. Exp. Ther.* 125:237–240 (1959).
16. E. R. Sigmund, R. Cadmus, and G. Lu. *Proc. Soc. Exp. Biol. Med.* 95:729–731 (1957).
17. H. O. J. Collier, L. C. Dinneen, C. A. Johnson, and C. Schneider. *Br. J. Pharmacol. Chemother.* 32:295–310 (1968).
18. J. Jacob. In P. Mantegazza and F. Piccinini (eds.), *Methods in Drug Evaluation,* North-Holland, Amsterdam, 1966, pp. 278–296.
19. P. L. Wood. In M. Kuhar and G. Pasternak (eds.), *Analgesics: Neurochemical, Biobehavioral and Clinical Perspectives,* Raven Press, New York, 1984, pp. 175–194.
20. R. I. Taber, D. D. Greenhouse, J. K. Rendell, and S. Irwin. *J. Pharmacol. Exp. Ther.* 169:29–37 (1969).
21. N. Kokka and A. S. Fairhurst. *Life Sci.* 21:975–980 (1977).
22. H. I. Chernov, D. E. Wilson, F. Fowler, and A. J. Plummer. *Arch. Int. Pharmacodyn.* 167:171–178 (1967).
23. R. Okun, S. C. Liddon, and L. Lasagna. *J. Pharmacol. Exp. Ther.* 139:107–109 (1963).
24. L. Vyklicky. In J. J. Bonica and D. Albe-Fessard (eds.), *Adv. Pain Res. Ther., Vol. 3,* Raven Press, New York, 1979, pp. 727–745.
25. N. Taira, K. Nakayama, and K. Hashimoto. *Tokohu J. Exp. Med.* 96:365–377 (1968).
26. F. Guzman, C. Braun, and R. K. S. Lim. *Arch. Int. Pharmacodyn.* 136:353–384 (1962).
27. K.-I. Adachi and Y. Ishii. *J. Pharmacol. Exp. Ther.* 209:117–124 (1979).
28. G. Deffenu, L. Pergrassi, and B. Lumachi. *J. Pharm. Pharmacol.* 18:135–138 (1966).
29. G. F. Blane. In A. Soulairac, J. Cahn, and J. Charpentier (eds.), *Pain,* Academic Press, London, 1968, pp. 218–222.
30. W. R. Martin. *Pharmacol. Rev.* 35:283–323 (1984).
31. M. Satoh, S. I. Kawagiri, M. Yamamoto, H. Makino, and H. Takagi. *Life Sci.* 24:685–690 (1979).
32. P. W. Beck and H. O. Handwerker. *Pflugers Arch.* 347:209–222 (1974).
33. N. Fjallbrant and A. Iggo. *J Physiol. (Lond.)* 156:578–590 (1961).
34. D. Armstrong, R. M. L. Dry, C. A. Keele, and J. W. Markham. *J. Physiol. (Lond.)* 120:326–351 (1953).
35. D. G. Teiger. *J. Pharmacol. Exp. Ther.* 197:311–316 (1976).
36. Z. S. Herman and W. Felinska. *Pol. J. Pharmacol. Pharm.* 31:605–608 (1979).
37. D. Dubuisson and S. G. Dennis. *Pain* 4:161–174 (1977).
38. M. A. North. *Life Sci.* 22:295–302 (1977).
39. P. L. Nielsen. *Acta Pharmacol. Toxicol.* 18:10–22 (1961).
40. D. Romer. In A. Soulairac, J. Cahn, and J. Charpentier (eds.), *Pain,* Academic Press, London, 1968, pp. 165–170.

41. G. Paalzow and L. Paalzow. *Acta Pharmacol. Toxicol.* 32:22–32 (1973).
42. C. Vidal, G. Girault, and J. Jacob. *Brain Res.* 233:53–64 (1982).
43. K. A. Bonnet and K. E. Peterson. *Pharmacol. Biochem. Behav.* 3:47–55 (1975).
44. W. O. Evans. *Psychopharmacologia* 2:318–325 (1961).
45. J. P. Rosenfeld and B. S. Holzman. *Brain Res.* 124:367–372 (1977).
46. T. L. Yaksh, J. C. Yeung, and T. A. Rudy. *Brain Res.* 114:83–103 (1976).
47. L. A. Dykstra. *J. Pharmacol. Exp. Ther.* 211:722–732 (1979).
48. J. L. Bloss and D. L. Hammond. *J. Pharmacol. Exp. Ther.* 235:423–430 (1985).
49. C. G. Lineberry. In W. I. Gay (ed.), *Methods of Animals Experimentation,* Academic Press, New York, 1981, pp. 237–311.
50. B. Weiss and V. C. Laties. *J. Pharmacol. Exp. Ther.* 143:169–173 (1964).
51. T. J. Crowley, A. J. Stynes, M. Hydinger, and I. C. Kaufman. *Arch. Gen. Psychiat.* 31:829–838 (1974).
52. S. Holtzman and J. Villarreal. *Psychon. Soc.* 17:161–162 (1969).
53. J. L. Malis. In H. W. Kosterlitz, H. O. J. Collier, and J. E. Villarreal (eds.), *Agonist and Antagonist Actions of Narcotic Analgesic Drugs,* University Park Press, Baltimore, 1973, pp. 106–109.
54. J. Pearl, C. R. Michel, and E. A. Bohent. *Psychopharmacologia* 14:266–270 (1969).
55. J. H. Chin and E. F. Domino. *J. Pharmacol. Exp. Ther.* 132:74–86 (1961).
56. C. L. Mitchell. *J. Pharmacol. Exp. Ther.* 146:1–6 (1964).
57. Y. Shigenaga, S. Matano, K. Okada, and A. Sakai. *Brain Res.* 63:402–407 (1973).
58. M. Skingle and M. B. Tyers. *J. Pharmacol. Method.* 2:71–80 (1979).
59. B. Matthews and B. N. Searle. *Pain* 2:245–251 (1976).
60. F. Greenwood, H. Horiuchi, and B. Matthews. *Arch. Oral Biol.* 17:701–709 (1972).
61. J. L. Oliveras, F. Redjemi, G. Guilbaud, and J. M. Besson. *Pain* 1:139–145 (1975).
62. P. Mason, A. Strassman, and R. Maciewicz. *Brain Res. Rev.* 10:137–146 (1985).
63. G. F. Steinfels and L. Cook. *J. Pharmacol. Exp. Ther.* 236:111–117 (1986).
64. M. Von Frey. *Abh. Ges. Wiss. Gottingen* 40:175–266 (1897).
65. F. Haffner. *Dt. Med. Wschr.* 55:731–733 (1929).
66. C. Bianchi and I. Franceschini. *Br. J. Pharmacol.* 9:280–284 (1954).
67. A. F. Green, P. A. Young, and E. I. Godfrey. *Br. J. Pharmacol.* 6:572–585 (1951).
68. C. A. Winter and L. Flataker. *J. Pharmacol. Exp. Ther.* 150:165–171 (1965).
69. L. O. Randall and J. J. Selitto. *Arch. Int. Pharmacodyn.* 111:409–419 (1957).
70. L. Rios and J. J. C. Jacob. *Life Sci.* 31:1209–1212 (1982).
71. L. Rios and J. J. C. Jacob. *Eur. J. Pharmacol.* 96:277–283 (1983).
72. H. O. J. Collier. In C. A. Keele and R. Smith (eds.). *The Assessment of Pain in Man and Animals,* Livingstone, London, 1962, pp. 262–270.
73. A. W. Duggan, B. T. Griersmith, P. M. Headley, and J. B. Maher. *Exp. Neurol.* 61:471–478 (1978).
74. F. E. D'Amour and D. L. Smith. *J. Pharmacol. Exp. Ther.* 72:74–79 (1941).
75. O. L. Davies, I. Raventos, and A. L. Walpole. *Br. J. Pharmacol.* 1:255–264 (1946).
76. M. R. Fennessy and J. R. Lee. In S. Ehrenpreis and A. Neidle (eds.), *Methods in Narcotic Research,* Marcel Dekker, New York, 1975, pp. 73–98.
77. W. D. Gray, A. C. Osterberg, and T. I. Scuto. *J. Pharmacol. Exp. Ther.* 172:154–162 (1970).
78. B. D. Appelbaum and S. G. Holtzman. *Life Sci.* 36:1069–1074 (1985).
79. K. Ramabadran and M. F. Jen. *Arch. Int. Pharmacodyn.* 274:180–188 (1985).
80. G. G. Berntson and J. M. Walker. *Brain Res. Bull.* 2:157–159 (1977).
81. B. C. Yoburn, R. Morales, D. D. Kelley, and C. Inturrisi. *Life Sci.* 34:1755–1762 (1984).
82. M. F. Jen and J. Han. *Chinese Med. J.* 92:576–582 (1979).
83. M. D. Tricklebank and G. Curzon (eds.). In *Stress Induced Analgesia,* John Wiley and Sons, New York, 1984, pp. 185–189.
84. W. L. Dewey and L. S. Harris. In S. Ehrenpreis and A. Neidle (eds.), *Methods in Narcotic Research,* Marcel Dekker, New York, 1975, pp. 101–109.
85. D. J. Mayer, T. Wolfle, H. Akil, B. Carder, and J. C. Liebeskind. *Science* 174:1351–1354 (1971).
86. P. A. J. Janssen, C. J. E. Niemegeers, and J. G. H. Dony. *Arzneim. Forsch/Drug Res.* 13:502–507 (1963).
87. M. Grotto and F. G. Sulman. *Arch. Int. Pharmacodyn.* 165:152–159 (1967).
88. R. D. E. Sewell and P. S. J. Spencer, *Neuropharmacology* 15:683–687 (1976).
89. D. Luttinger. *J. Pharmacol. Method.* 13:351–357 (1985).
90. G. Woolfe and A. D. MacDonald. *J. Pharmacol. Exp. Ther.* 80:300–307 (1944).
91. N. B. Eddy and D. Leimbach. *J. Pharmacol. Exp. Ther.* 107:385–393 (1953).
92. J. Jacob and M. Blozovski. *Arch. Int. Pharmacodyn.* 133:296–309 (1961).
93. J. Jacob, C. Lafille, G. Loiseau, P. Echinard-Garin, and C. Barthelemy. *L'encephale* 4:1–16 (1964).
94. J. Jacob, G. Loiseau, P. Echinard-Garin, C. Barthelemy, and C. Lagille. *Arch. Int. Pharmacodyn.* 148:14–30 (1964).
95. J. Jacob, E. C. Tremblay, and M. C. Colombel. *Psychopharmacologia (Berlin)* 37:217–223 (1974).
96. J. J. C. Jacob and K. Ramabadran. *Eur. J. Pharmacol.* 46:393–394 (1977).
97. J. J. C. Jacob and K. Ramabadran. *Br. J. Pharmacol.* 64:91–98 (1978).
98. R. C. A. Frederickson, V. Burgis, and J. D. Edwards. *Science* 198:756–758 (1977).
99. P. Grevert and A. Goldstein. *Psychopharmacologia* 53:111–113 (1977).
100. J. Jacob, K. Ramabadran, J. M. Girault, C. Suaudeau, and G. Michaud. In J. M. Van Ree and L. Terenius (eds.), *Characteristics and Functions of Opioids,* North-Holland, Amsterdam, 1978, pp. 171–172.
101. J. P. O'Callaghan and S. G. Holtzman. *J. Pharmacol. Exp. Ther.* 192:497–505 (1975).
102. C. Barthelemy, E. Tremblay, and J. Jacob. *J. Pharmacol. (Paris)* 2:37–52 (1971).
103. J. Jacob and C. Barthelemy. *Ann. Anesth. Franc.* 9:197–212 (1968).
104. K. Ramabadran and J. J. C. Jacob. *Arch. Int. Pharmacodyn.* 236:27–42 (1978).
105. K. Ramabadran and J. Jacob. *J. Pharmacol. (Paris)* 11:84–85 (1980).
106. J. J. C. Jacob, K. Ramabadran, J. C. Rousselle, and P. E. Chabrier. In E. Leong Way (ed.), *Exogenous and Endogenous Opiate Agonist and Antagonists,* Pergamon Press, New York, 1980, pp. 99–102.
107. K. Ramabadran and J. J. C. Jacob. *Jap. J. Pharmacol.* 32:1059–1065 (1982).
108. K. Ramabadran, G. Michaud, and J. J. C. Jacob. *Ind. J. Exp. Biol.* 20:74–76 (1982).
109. K. Ramabadran. *Eur. J. Pharmacol.* 98:425–427 (1984).
110. K. Ramabadran. *Jap. J. Pharmacol.* 37:296–299 (1985).
111. P. F. VonVoigtlander. In D. Lednicer (ed.), *Central Analgetics,* John Wiley and Sons, New York, 1982, pp. 51–79.
112. A. Carmon and R. Frostig. *Life Sci.* 29:11–16 (1981).
113. M. C. Lombard, B. S. Nashold, D. Albe-Fessard, N. Salman, and C. Sakr. *Pain* 6:163–174 (1979).
114. A. I. Basbaum. *Exp. Neurol.* 42:490–501 (1974).
115. L. S. Anderson, R. G. Black, J. Abraham, and A. A. Ward, Jr. *J. Neurosurg.* 35:444–452 (1971).
116. A. I. Basbaum and P. D. Wall. *Brain Res.* 116:181–204 (1976).
117. A. W. Pircio, C. T. Fedele, and M. E. Bierwagen. *Eur. J. Pharmacol.* 31:207–215 (1975).