Review Article

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A Review on Analgesic Drugs

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Abstract

Pain is composite distressing sensory experiences that include several elements like time, space, intensity, emotion, perception and motivation. Analgesics are the Pharmacological agents; acts selectively for relieve pain by acting on the central nervous system or peripheral pain biochemical interactions without causing more effect on consciousness. Analgesics may come under narcotic or non-narcotic category. The evolutionary study of pain on animals arises ethical, philosophical and technical problems. Philosophically, theproblem of study the pain on animals is that pain cannot be observed directly in animals but can only be accessed by measuring the animal responses to nociceptive stimuli. The perceive reactions are always motor responses varyingfrom spinal reflexes to complex behavior. The animal models hired for screening of analgesic agents, include Pain state models depends thermal stimuli, mechanical stimuli, electrical stimuli and chemical stimuli as mean of accessing. The neuronal basis of most of the above laboratory models can't deeply explained, however their usage is profitable in predicting analgesic activity of newly discovered chemical agents.

Keywords: Analgesics; Thermal; Mechanical; Chemical.

INTRODUCTION

- Pain[1] is derived from Greek "Poin" meaning penalty.
- Derived from Latin "Poena" meaning Punishment from God.
- Pain is composite distressing sensory experiences that include several elements like time, space, intensity, emotion, perception and motivation.
- An analgesic or pain relieving agent is any elementof the group of drugs used to achieve analgesia relief from pain.
- Analgesics are the Pharmacological agents; acts selectively for relieve pain by acting on the central nervous system or peripheral pain biochemical interactions without causing more effect on consciousness.

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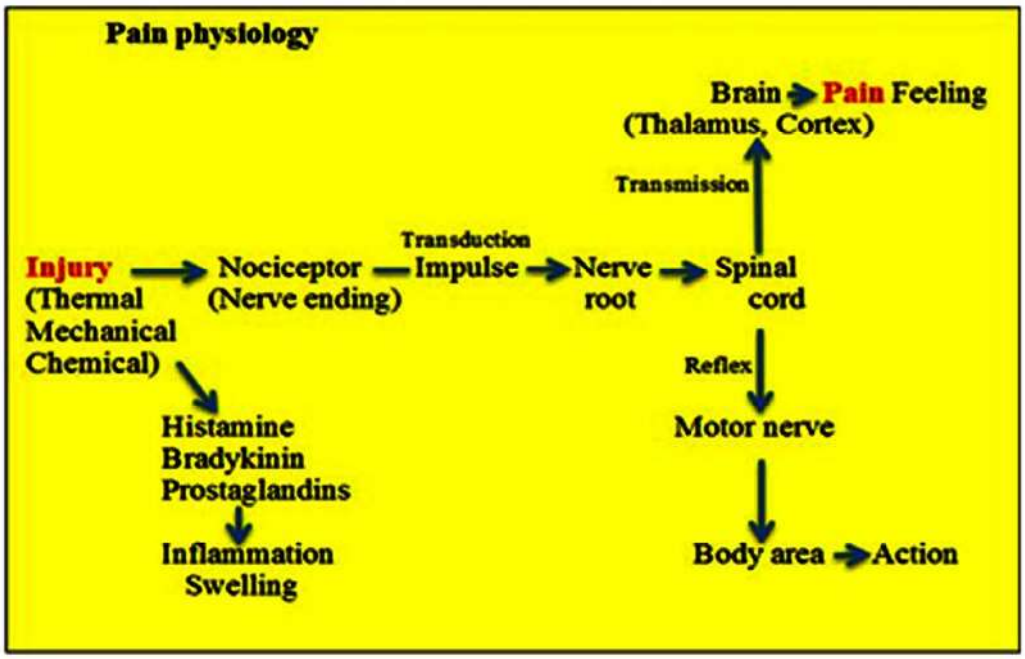


Fig. 1: Pain physiology

PHASES OF PAIN[2-5]

Phases I (Acute nociceptive pain)

- Brief noxious stimulifairly simple & direct route centrally towards the thalamus & cortex conscious perception of pain possibility for modulation synaptic relays along the way.
- Close correlation the discharges in peripheral nociceptors & subjective expression pain.

Phase 2 (Inflammatory Pain)

- Harmful stimulus highly severe or extend tissue damage and inflammation.
- Raised activity and reactiveness of sensitized nociceptors.
- Larger afferent inflow from injured area to CNS
- Nociceptive neurons present in spinal cord change their responsiveness.

Phase 3 (Neuropathic Pain)

- Indications of neurological disease.
- Lesions to the peripheral nerves or hurt to any portion of somatosensory system present in the CNS.
- Spontaneous pain, set off by non dangerous stimuli or are overemphasize responses to small noxious stimuli.
- Probable reason
  - ❖ Pathological alterations in damaged neurons.
  - ❖ Reactive changes happensas response to nociceptive afferent input and also to damage of some portions of the normal afferent inputs.

METHODS OF PAIN CONTROL[6-9]

- Removing the cause.
- Jamming the pathway of painful impulses.
- Raising the pain threshold.
- Preventing pain response through cortical depression.
- With the aid of psychosomatic methods.

Removing the Cause

- It is the pleasing method of scheming pain.
- If it is virtuoso, the environmental alteration in tissue would be abolished.
- Free nerve endings would not be agitated and no impulses would be start off.
- This method concentrated affects pain insight.

Blocking the Alleyway of Painful Impulses

- It is the widely worn method in dentistry for

controlling pain.

- A proper drug, having local anesthetic activity is injected into the tissues in immediacy to the nerves involved.
- It ceases the depolarization of the nerve fibers at the area of drug absorption, thus averting those fibers to conducting any impulses beyond that point.
- This method of pain control is feasible by obstructing with pain perception.

Raising the Pain Threshold

- It is based on the pharmacologic actions of the drugs having analgesic properties.
- These drugs increase the pain threshold centrally and therefore hinder with pain reaction.
- The root of the organic stimulus may still be there.
- Pain insight is impassive, but pain reaction is diminished and thus pain reaction threshold is increased, but it is impossible to remove all pain of the most intense nature.

Averting Pain Reaction Through Cortical Depression

- Abolishing pain by this method is through general anesthesia and general anesthetic agents.
- The agent, by raising depression of the CNS, ceases any conscious action to a painful stimulus.

Averting Pain Reaction with the Aid of Psychosomatic Methods

Relaxation Training

Relaxation involves slow and profound breathing to discharge tension from muscles and mitigate pain.Achieving good relaxation needs practice, but relaxation training can stress on alertness away from pain and discharge tension from all muscles. Relaxation audio-visuals are widely available to help you learn these skills.

Bio-Feedback

Bio-feedback is taught byexperts who utilizeparticular machines to assist you learn to manage bodily functions like heart rate and muscle tension. As you learn to discharge muscle tension, the machine instantaneously indicates success. Bio-feedback can be helpful by support relaxation training. Once the practice is learned successfully, it can be adept without the aid of the machine.

Visual imagery and distraction

CLASSIFICATION[10-15]

Analgesics are classified as:

- Narcotics
  - ❖ Acts centrally
  - ❖ Cause addiction
  - ❖ Produce CNS depression
  - ❖ Do not produce gastric irritation
  - ❖ Show no anti-inflammatory effect
  - ❖ Ex. Morphine, Pethadine, Fentanyl
- Non-narcotics
  - ❖ Act peripherally
  - ❖ Do not cause addiction
  - ❖ Do not produce CNS depression
  - ❖ Produce gastric irritation
  - ❖ Show anti-inflammatory effect

  Ex. NSAID

Mechanism of Action

- Analgesic drugs act by several ways on the peripheral and central nervous systems.
- Opioids create analgesia by fastening to specific G – protein coupled receptors in brain and spinal cord.
- NSAIDs inhibit the activity of both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) and cease the synthesis of prostaglandins and thromboxanes.
- Inhibition of COX-2 causes the anti-inflammatory, analgesic and antipyretic effects.

SCREENING MODELS[16-24]

Pain State Models using Chemical Stimuli

- ➢ Acetic acid induced writhing test
- ➢ Stimulation of hollow organs
- ➢ Formalin test
- ➢ Randal sellito test
- ➢ Intra arterial Bradykinin

Pain State Models using Electrical Stimuli

- Electrical stimulation of the tail
- Grid-shock test (Flinch-jump test)
- Stimulation of the tooth pulp
- Monkey-shock titration test
- Stimulation of the limbs

Pain State Models using Mechanical Stimuli

- Heffner's tail clip test in mice
- Strain gauges
- Von-Frey filaments
- Inclined plane test

Pain State Models using Thermal Stimuli

- The tail-flick model using radiant heat
- Immersion of the tail in warm water
- Hot plate test
- Paw-withdrawal test
- Pain state models using cold stimuli

Writhing Tests

Purpose and rationale

- Pain is created in the animal by injecting irritants like acetic acid into peritoneal cavity of mice.
- The animals respond with feature stretching behavior which is writhing.
- The test is appropriate to notice analgesic activity of peripherally acting drugs.

Procedure

- Swiss Mice (20-25g) are elected and separated into standard, test & control group (6 animals) correspondingly.
- Suitable volume of acetic acid solution is administered to the mice (control group) and positioned individually in the glass jar.
- The commencement of writhing, abdominal contractions & trunk twist response are write down for 10 min.
- The test and standard drug is administered 15 min prior to the acetic acid administration.
- Drugs to be taken are.
  - ❖ Phenylbenzoquinone in a concentration of 0.02% is suspended in a 1% suspension of carboxymethyl cellulose. (Dose – 0.25ml i.p.)

OR

  - ❖ 0.1ml of 0.6% solution of acetic acid (i.p.)

Phenylbenzoquinone solution is generally less used because of its stability problem (solubility, oxidation, photosensitivity etc).

Evaluation

- The writhing period is recorded and compared with the control group.
- Writhing response in the drug treated must be less when compared to the acetic acid treated control.

$$\% \text{ inhibition} = \frac{(\text{Average writhers in control group} - \text{average writhes in treated group} \times 100)/}{(\text{Average writhers in control group})}$$

Many modifications are done for this test that is replacement of acetic acid with that of phenylbenzoquine and acetylcholine. And also the changes in the experimental conditions as well as monitoring to increase the sensitivity of the test.

Stimulation of Hallow Organs

- In this test true visceral pain can be produced in animals by injecting algogenic substances directly into hallow organs.

For example

- ❖ Administration of formalin into the rat colon can produce complex biphasic type of "pain behaviour" involving initial phase of body stretching and contraction of either the flanks or the whole body and a second phase that predominately involves abdominal licking and nibbling.
- ❖ Intra-colonic infusion of glycerol also produces abdominal constrations.

Formalin Test

Principle

The formalin test in rats has been proposed as a chronic pain model, which is sensitive to centrally active analgesic agent.

Procedure

- Take male Wistar rats of weighing in between 200 – 300g.

- Injection 0.05 ml of 10% formalin into the dorsal surface of the forepaw of rat.
- Test or standard drug administered simultaneously either s.c. or orally.
- Each rat is placed individually in a clean, transparent, polypropylene cage for observation.
- Response: elevation and licking of the paw.
- Readings are taken at 30 and 60 mins after formalin injection and scored according to the following scale:
  - **0** – full weight placed on the paw.
  - **1** - the injected paw rests lightly on the floor and bears no weight.
  - **2** – when the injected paw is selectively elevated and is not in contact with any other surface.
  - **3** – when the paw is licked or bitten by the animal.
- Analgesic response or protection is indicated when both the paws are resting on the floor with no obvious favoring of the injected paw.

Randal-Sellito Test

Principle:

The main principle involved in this is inflammation enhances the sensitivity to pain which can be modified by analgesics.

In this method, Brewer's yeast is used for producing inflammation which in-turns increases the sensitivity to pain.

Equipment

Randall-sellito test equipment (which is a small equipment with projection pin).

Selection of animal

Before conducting the test, the animals are subjected to the pain. Select only those animals whose pain threshold is less than 80 g.

Procedure

- Take male Wistar rats of weight 150 – 200 g and allow group 6 – 10 rats in a group.
- Inject 0.1ml of a 20% suspension of Brewer's yeast in distilled water subcutaneously into the plantar surface of the left hind paw of the rat.
- Three hours later to the experiment, pressure is applied through the tip of plantar surface of the rat's foot at a constant rate by a special apparatus to the point at which the animal starts struggling, squealing or attempting to bite.
- The tests are done at 15 mins intervals and pain threshold values are recorded.
- The time at which the greater increase in pain threshold is recorded and is considered as time of peck effect.
- For recording the dose response, the pain threshold is recorded at 0hrs and again at the predetermined peak time.
- The mean applied force is determined for each time interval tested.

$$\text{Percentage increase in pain threshold} = \frac{\text{(Applied force for vehicle treated rats – applied force for control rats} \times 100)}{\text{(Applied force for vehicle treated rats)}}$$

- Statistical compare the test and standard with that of control to find out potency and LD50 the compound (test).

Intra-Arterial Bradykinin Test

Principle

Intra-arterial injection of bradykin in evokes vocalization and induction of pain in experimental animals which has been used for the evaluation of analgesic drugs.

Procedure

- Take male Wistar rats of weighing around 300g, group 10 rats in group.
- Lightly anaestatized rats with the ether.
- Thereafter, a polyethylene catheter having an internal diameter of 0.5mm is inserted centripetally into the right carotid artery.
- The catheter is then passed through the S.C. tissues to protrude from the back of the animal.
- One hour after recovery from anaesthesia, the first dose of bradykinin (0.05 – 0.1ug/kg) is injected through the catheter which results in dextrorotatory movements of head, flexion of the respective forelimb and an occasional squeaking.
- Repeated administration of bradykinin at regular interval does not lead at tachyphylaxis.
- For each rat, the minimum dose of bradykinin

necessary to produce these effects is determined.

- Once the sensitivity of an individual rat has been established, a dose of test compound is administered and their effects on the bradykinin responses are scored.
- The bradykinin injections are replaced at 5 mins intervals until the analgesic response to bradykinin reappears.
- Each rat receives one drug at one dose level.
- The criterion for protection is disappearance of bradykinin effect after at least two consecutive doses of bradykinin.

Electrical Stimulation of the Tail

Principle

Electrical stimulation of the tail through intracutaneous needles in animal produces consistent responses.

Three types of pain thresholds are determined following the electrical shock applied to the tail corresponding to 3 different levels of integration of pain within the CNS:

- A low density stimulation produces a motor response, tail withdrawal (which is a low grade spinal reflex).
- A higher voltage induces a simple vocalization involving the lower brain stem.
- A stimulation using higher voltage produces a brief vocalization after the stimulation is terminated (vocalization after discharge) representing the affective component of pain response involving hypothalamus and rhinencephalon.

Procedure

- Take male Swiss mice with an average weight of 20 g.
- Place the mice into special cages.
- A pair of alligator clips is attached to the tail whereby the positive electrode is placed at the proximal end of the tail.
- Rectangular wave pulses from a constant voltage stimulator at an intensity of 40 – 50 V are applied.
- The frequency of stimulation is 1 shock per second and the pulse duration 2.5 minutes.
- The normal response time range of stimuli is 3 – 4 s.
- Following administration of the test drug, the response time is registered at 15 mins interval until the reaction time returns to control levels.
- The data for each animal are plotted with reaction times on the ordinate and time intervals following administration on the abscissa. The area under the time response curve is calculated.

In control animals, the reaction time remains fairly constant and the area under curve is approximately zero.

Grid-Shock Test (Flinch-Jump Test)

Purpose and Rationale

- The electric grid shock test in mice has been explained by Blake *et al.*
- The analgesic properties of drugs like Morphine, Acetylsalicylic acid can be estimated by the Flinch jump response of rats.

Procedure

- Male mice (18-20 g) are chosen and placed individually in plastic chamber.
- The floor of the box is wired with stainless steel wire.
- The incentive is given in the form of square wave pulses (30 cycles per second).
- The output of stimulator is coupled to alternate wires of grid.
- The fixed resistance is located with the grid & parallel to an oscilloscope to permit calibration in mill amperes.
- With increase in shock intensities the mice flinch, demonstrate startling reaction & increase locomotion or attempt to jump.
- The behavior is exactly reflected on the oscilloscope by marked fluctuations of the displayed pulse.
- Pain thresholds are measured in each individual mouse twice before & after the administration of the test drug.

Evaluation

- The current determined in milli-amperes is recorded for each animal before and after administration of the drug.
- The average pain threshold values for each group at each time interval are measured and statistically compared with the control values.

Stimulation of the Tooth Pulp

Principle

- Electric stimulation of the tooth pulp has been worn in antinoceptive screening and is a useful model for studying facial pulp. Stimulation of tooth pulp produces characteristic painful reactions such as licking, biting, chewing and head flick which can be observed easily.

Procedure

- Take rabbits of either sex of weighing in between 2 – 3 kgs and group 8 – 10 animals in a group.
- And anaesthetised the animals by 15mg/kg of thiopentone sodium or 0.2mg/kg fentanyl citrate administered i.v.
- Pulp chambers are exposed close to the gingival line in the lateral margins of the two front upper incisions with a high speed dental drill.
- The clamping electrodes are placed into the drilled holes during the experiment.
- After acclimatization period of 30 mins, stimulation is started to determine the threshold value.
- The stimulus is applied by rectangular current with a frequency of 50 Hz and duration of the stimulus of 1s.
- The electrical current is started with 0.2mA and gradually increased until the phenomenon of licking occurs.
- For assessing the basic value, the threshold is determined 3 times in each animal and each animal serves as its own control.
- The test substance is administered either i.v. or by oral route.
- The threshold of anti-nociceptive effect is determined at 0, 15, 30, 60 and 120 min.

Evaluation

Drugs providing the effects between the ranges of 10 – 90% are used.

The anti-nociceptive effect is defined as the increases of the threshold versus the initial control by a factor of 2 or more.

Central analgesics have been found to be very effective in this test. Drugs like ketamine and NSAIDs also give positive response with this model.

MONKEY-SHOCK TITRATION TEST

Procedure

- In this model, the monkeys are placed in restraining chairs.
- Electrical current is delivered by a Coulbourn Instrument programmable shocker through electrodes coupled to two test tube clamps, which are connected to a shaved portion of the tail.
- The current ranges from 0 – 4 mA through 29 progressive steps.
- The monkey presses a bar to cut short the shock.
- A stable baseline shock level is created for each monkey on the day prior to drug administration.
- After drug administration, shock titration activity is evaluated according to the change in maximum level of median shock intensity attained for drugs as compared to control levels.
- Doses of 3.0 mg/kg i.m. morphine, 1.7 mg/kg i.m. methadone and 10 mg/kg i.m. pentazocine were established to be effective.
- The monkey shock titration test may be helpful for final determination of a new compound before administration to man.

Stimulation of the Limbs

Principle

Electromyographic recordings of nociceptive limb reflexes have been used for pharmacological studies of nociception, but they are far less common than behavioral tests. These electromyographic studies have allowed the quantification of responses regardless of whether there is any movement.

HEFFNER'S TAIL CLIP TEST IN MICE

Principle

This method is based on the observation of Haffnee (1929) that the raised (straub's) tail in mice treated with morphine to be less sensitive to noxious stimuli.

Procedure

- Take male Swiss mice of weighing between 20 - 25 g and divided the animals into groups which contains 10 animals in each group.
- The test compounds are administered

subcutaneously fed mice or orally to fasted mice.

- The drug is administered 15, 30 or 60 min prior to testing.
- An artery clip is usedon the root of the tail (approximately 1cm from the body) to bring pain.
- The animal speedily responds to this noxious stimulus by biting the clip or the tail close to the location of the clip.
- The time between onset of stimulation and response is calculated by a stop watch of 0.1 sec.

Evaluation

- A cut-off time is measured by taking the average reaction time plus 3 times of the standard deviation of the combined latencies of the control mice at all time periods.
- Any reaction time of the lat animals which is better than cut off time is called a positive response indicative of analgesic activity.
- The length of time until response indicates the periods of greatest activity after dosing.

Another method of mechanical stimulation is the tail compression test. In this candal compression test, the stimulation 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 by 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.

The tip of the tail is most suitable for obtaining a prompt response.

Tail Flick Model (Radiant heat Method)

Purpose and Rationale

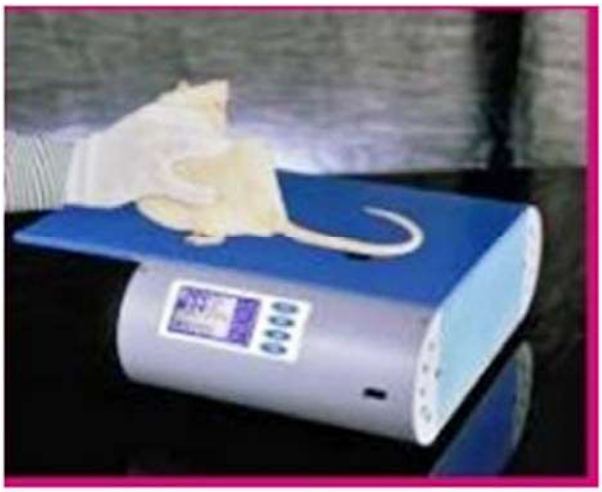
- The tail flick test with radiant heat is a simplified method.
- The use of thermal radiation to the tail of an animal leads the withdrawal of tail.
- The morphine like drugs is efficient of prolonging the reaction time.

Procedure

- Wistar rats (170-210 g) are chosen and divided into standard, test & control group respectively.
- Proper temperature is maintained on the radiant source.
- The tail of the rat is positioned on the radiant source & time taken for the rat to withdraw its tail is noted.
- Usually withdrawal time is within 2-10 sec.
- The Tail-flick latency is noted before & after the administration of standard or test compound.

Evaluation

- The tail flick latency in the test, standard and control animals is compared.
- Using various doses ED50 values can be measured.



Immersion of the Tail in Warm Water

Principle

- The rodent tail withdrawal reflux can be elicited by immersion of the tail in warm water at 550C. This test is specific for opiod like central analgesics and is used to differentiate them peripheral analgesics.

Procedure

- Take young female Wistar rats of weighing between 150 – 200 g.
- Place the rats into individual cylindrical rat holders leaving the tail hanging out freely.
- The animals are allowed to get acclimatized to rat holders for 30 mins before testing.
- The lower 5 cm portion of the tail is marked.
- This part of the tail is immersed in a cup of freshly filled water at exactly 550C temperature.
- The reaction time is recorded using a stopwatch of 0.5 sec accuracy. After each determination the tail is carefully dried.
- The reaction time is determined before and periodically 0.5,1,2,3,4 and 6 hrs after the oral

or S.C. administration of the test substance. The cut off time of immersion is 15 sec.

- The withdrawal time of control rats usually lies between 1 and 5.5 sec.
- A withdrawal time higher than 6s is considered as a positive analgesic response.

Hot-Plate Test

Purpose and Rationale

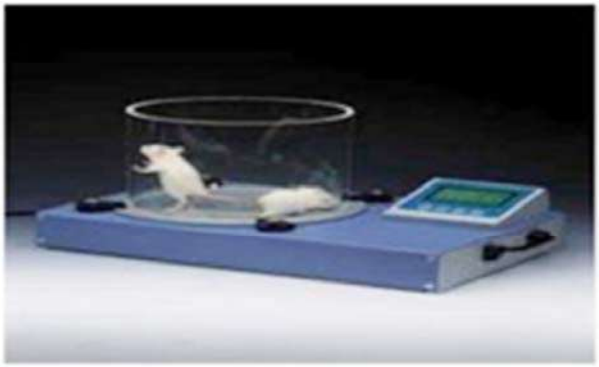
- The paws of mice and rats are sympathetic to heat at temperatures which are not destructive to skin.
- The reactions for heat application are jumping, withdrawal of the paws and licking of the paws.
- The reactions are extended after injecting of centrally acting analgesics, while peripheral analgesics of the acetylsalicylic acid or phenyl-acetic acid type do not normally concern these responses.

Procedure

- Groups of 10 mice (18-22 g) are chosen and divided into standard, test & control group in that order.
- The temperature of the hot plate is set at 55° to 56°C.
- The animals are positioned on the hot plate & time for either licking or jumping occurs is recorded.
- The latency is noted before & after 20, 60 and 90 min after the administration of standard or test drugs.

Evaluation

- The extension of latency time is compared between the test, standard and control animals.
- Using several doses ED50 values can be measured.



PAW-WITHDRAWAL TEST[25]

This test is completelysimilar to the test of D'Amour and Smith (1941) but facilitates with the advantage that it does not concerned the pre-eminent organ of thermoregulation in rats and mice, i.e., the tail.In this test, radiant heat is applied on a paw that had inflamed by a subcutaneous injection of carrageenin before the process. Not only by this way, can an inflammation also be induced by exposure to ultraviolet rays. One extreme benefit in this method is that heat is induced to a freely moving animal.

Pain State Models using Cold Stimuli

Cold stimuli are extremelyinfrequent used for test acute pain, but it is more general to test cold allodynia in animal models of neuropathies.

Differentiation of Peripheral and Central Effects of Analgesics

A variety of pain models are available to demonstrate the anti-nociceptive activity of drugs. In most of the models it is difficult to differentiate between the centrally acting and peripherally acting analgesic drugs.

Method used

Isolated perfused rabbit ear models.

Procedure

- Take male New Zealand rabbits of weight in between 3 – 4 kgs.
- Anaesthetized the rabbits with pentobarbitone sodium through i.v. route using an initial dose of 30mg/kg and thereafter maintained with additional doses as required.
- One ear was prepared in such a way that the only connection left to the animal's body from it was via the great auricular nerve.
- The isolated ear was perfused via the main artery with oxygenated tyrode solution (O_2:CO_2-95%:5%) prostaglandin E2 (PgE2) 40ng/ml at a temperature of 34°C and at a constant flow rate of 2 ml/min using a capillary pump.
- Infusion pressure and infusion temperature were monitored and recorded.
- • Bradykinin (2 – 4 micro gram/ml) depending on the responsiveness of the animal, and acetylcholine (100 microgram/ml) in tyrode buffer were injected into the perfusion system as 0.1ml aliquots at approximately 10

mins intervals.

- The system remains stable for 6 – 8 hours.
- Drugs were administered either locally in the perfusate or systematically by i.v. or i.p. injections.
- Systemic blood pressure was monitored by a pressure tranducer inserted into the carotid artery and linked to an electro-manometer with simultaneous recording.
- The head flick response of the facial musculature which is a parameter of pain response was recorded with an isometric strain guage connected by a cotton thread to the incisors of the rabbit.

The NSAIDs, paracetamol and morphine were tested by this method. It allows for the distinction between centrally and peripherally acting analgesic drugs. It was found that local aspirin, diclofenac sodium and paracetamol inhibited the pain reflexes induced by bradykinin whereas local morphine proved to be ineffective. On the contrary after systemic administration only morphine but not aspirin, diclofenac sodium and paracetamol were active in the therapeutic range. This model is, therefore, useful in differentiating peripheral from central sites of action of analgesic drugs.

CONCLUSION

Algesia implies experience related to pain, whereas hyperalgesia indicates increased sensitivity to pain. Analgesics are the agents, which selectively relieve pain by acting in the CNS or by peripheral pain mechanisms without significantly altering consciousness. Analgesics may be narcotic or non-narcotic. Non-narcotic analgesics[6] such as aspirin, ibuprofen, paracetamol etc. possess not only anti-inflammatory properties but also antipyretic activity besides analgesic activity. For many of them the mode of action has been elucidated as an inhibition of cyclooxygenase in the prostaglandin pathway. Nevertheless, new peripheral analgesics have to be tested not only for their in vitroactivity on cyclooxygenase, but also for their in vivo analgesic potential. The most commonly used methods for measuring peripheral analgesic activity are the writhing tests in mice (various modifications) and the *Randall Selitto test*[27] in rats. The study of pain in animals raises ethical, philosophical and technical problems. Philosophically, theproblem of studying the pain on animal models is that pain cannot be observed directly in animals but can only be accessed by measuring the animal responses to nociceptive stimuli. Technically, the perceive reactions are always motor responses varying from spinal reflexes to complex behavior. The animal models hired for screening of analgesic agents, include Pain state models depends thermal stimuli, mechanical stimuli, electrical stimuli and chemical stimuli as mean of accessing. Although, the neuronal basis for the above laboratory models is not clearly understood, even though their application is of massive value in predicting the pain relievingactivity of most of the pharmacological agents.

Conflict of Interests: Conflict of interest declared none.

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