Assays in analgesic studies

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Abstract: Pain is a multiplex uncomfortable experience consisting of sensory cases including emotion, time, cognition, time and motivation. It can be persistent, chronic or momentary i.e. lasting for a very short period of time and its location can either be muscular or visceral. Analgesics are molecules that particularly mitigate pain by acting on the peripheral pain mechanism or central nervous system without remarkably responsiveness.Different animals have been used as experimental models for the direct investigation and study of neuronal activity in animals which have been administered with anaesthesia using either invasive procedures or by the study of their behaviour.Someof problems including ethics, technics and philosophyare involved in the study of pains using different models.The purpose of this review is to give an overview about inducing pain in different types of animal tissues and this includes the pain-state model using the chemical, electrical, mechanical and thermal stimuli. Also some of the shortcomings of the methods are highlighted.

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1. Introduction

In health care diseases of all kinds are associated with pain and is one of the important symptoms that helps in the evaluation and diagnosis of a certain ailment. Characterization of pain depends on its cause, duration and location. The main causes of pain are usually of two types including inflammation of the affected body part or nerve injuries. Pain can be persistent, chronic or momentary i.e. lasting for a very short period of time. Location of the pain can be assessed to be either muscular or visceral (National Research Council, 2009). Several symptoms of underlying disease related to patient's cognition, psychology and social aspects may confuse the characterization of pain in clinical trials. Moreover, malaise and fever are commonly associated with most of the diseases and complicate the characterization of pain in humans. Such factors make it difficult to understand and assess pain in clinical trials. This problem can be overcome by the use of experimental pain models in which the researcher can control the intensity, duration, location, frequency and nature of the pain stimulus and can study the psychological and neurophysical responses (Drewes et al., 2003). Apart from being practically challenging, the experiments on human models are also ethically limited favouring the use of animal models for the study of pain.

Different animals have been used as experimental models for the direct investigation and study of neuronal activity in animals which have been administered with anaesthesia using either invasive procedures or by the study of their behaviour (Sengupta et al., 1994). Animal studies are however limited in a way because of the difference in neurobiology among different species which makes it harder for findings from the animal studies to be interpolated to humans. Apart from this, several multidimensional complex mechanisms are involved in producing the pain and these mechanisms involve several parts of central nervous system. Therefore, even though the reflexes of nociception recorded from the certain nervous system (CNS) pathways of animals form the basis of research, complex mechanism associated with the pain mechanisms of CNS are not properly understood. Moreover, the animal studies are not enough to completely express the pain experienced by man humans (Le Bars et al., 2001). Similarly, the lack of communication

with animals verbally as it can be done in case of human trials is an obstacle in the evaluation of pain and its characteristics. Animal models mostly depend on the change of response to a stimulus applied to the animal or the change is threshold. However, animal models can be used in finding out the several mechanisms associated with pain that change an animal's response to a given stimulus (Woolf et al., 2001).

Pain state models can be classified on the basis of their mechanisms, involved tissue and the time duration of the pain. Mechanisms involved are of two types; inflammatory and neuropathic while tissues involved can be viscera, muscles and skin. Similarly duration for pain can be either shorter or longer also termed as phasic and tonic respectively.Phasic pain lasts from milliseconds to seconds and it does not reach minutes in its duration while tonic pain is of longer duration and lasts for minutes. The purpose of this review is to give an overview about inducing pain in different animal tissues by using different pain models.

2. In vivo approaches to analgesic studies

Many approaches are employed for screening of analgesic molecules and stimulation of pain. These models include the pain-state model, in which pain can be induced by thermal stimulus, mechanical stimulus, electrical stimulus and chemical stimulus. Animal models such as rat, mouse, rabbit and monkey are used in these models.

Using humans as pain state models can serve as translators between clinical research and animals. They can also help in understanding the several animal mechanisms tested and can be evaluated in the healthy volunteers. This can be used for the predicting a specific drug efficacy in certain patients (Arendt-Nielsen et al., 2007). Nevertheless, this may not be achieved as the use of human as a pain state model may not be ideal to some of the techniques and chemicals that will be used. Neurophysiological or psychophysical methods are used for the output assessment from these pain state models (Gracely, 1999). Out of these two methods, psychophysical is considered to be the simplest way to estimate the response to pain stimulus (Schmelz, 2009). Psychophysical methods are based on the measurement of subjective pain experience as pain thresholds or by the use of standard scales. On the other hand, neurophysical methods are based on the measurement of nociceptive reflexes of withdrawal or brain potentials produced.

2.1 pain-state model through thermal stimuli

Heat can be an ideal stimulus for stimulating receptors on the cutaneous membrane. The nociceptive activation source can be away from its target in direct contact with skin layer. For instance, the heat of radiation from a lamp in direct contact with the skin layer. The heat from radiation has a unique stimulus for nociceptive receptors and has importance over other means of thermal stimulation because it gives no tangible stimulus (Milind and Monu, 2013). Hence, heat is the suitable stimulus for activating cutaneous receptors (Saleem and Naz, 2017).

2.1.1 The immersion of the tail in hot water/ tail-flick model using radiant heat

This model is one of the unique methods in human subjects exposed to radiant heat (Hardy et al., 1940). This model was used in rat by Hardy and his co-researchers. After the tail of experimental animal was subjected to thermal radiation, the animal moved its tail in a simple dynamic motion (Hardy et al., 1957; Smith et al., 1943). The method of movement of the tail from the source of heat is known as tail-flick latency. In this procedure, at the time of application, a timer is started and the time taken for the experimental rat to withdraw its tail from the source of heat is observed and recorded. The time of withdrawal for rats is normally within 2 to 10 seconds. It is recommended that the time of exposure of the rat should not exceed 20 seconds to prevent injury to the tail of the animal. A rheostat in the apparatus regulates the amount of current passing through the filament. Some researchers have substituted the hot with cold stimuli and also, in place of rats, monkeys can be used in this procedure. The variance of the procedure describe above can be the use of tail immersion (Parle et al., 2013; Saleem and Naz, 2017). From some scientist opinion, the procedure of tail-flick model using radiant heat is majorly ideal for showing the analgesic properties of opioid but not for the partial agonists of opioid. This opinion was based on pharmacological point of view. In addition, when the distal part of the tail is stimulated, the procedure is more sensitive to morphine than when the proximal part of the tail is stimulated and an intermediate effect is recorded on the middle part of the tail (Milind and Monu, 2013).

The test is very ideal for selecting analgesics with morphine features and it does not need some unique skills. Also, the method is less time consuming and the outcomes are quite accurate (Milind and Monu, 2013). However, the method also has some limitations. The process of habituation may occur during the tail-flick test and consistent is not observed in repetitive

stimulation. The process of habituation is seen along with elevated heat intensity and reduced interval of inter-stimulus. The tail-flick method is not suitable for opioid partial agonist and it is recommended the exposure of the rat's tail to radiant heat over 20 seconds to avoid injury to the skin of the tail (Milind and Monu, 2013).

2.1.2 Paw-withdrawal procedure

In this test, the pre-eminent organ (organ) of thermoregulation in mice and rats are not involved and it is similar to the method of D'Amour and Smith (D'Amour and Smith, 1941; Hargreaves et al., 1988). In this method, swollen paw activated by injection of subcutaneous carrageenan is exposed to radiant heat to cause inflammation. Ultraviolet rays can also be used to produce inflammation (Perkins et al., 1993). An important advantage of this procedure is that radiant heat is exposed to a free moving animal (Perkins et al., 1993).

2.1.3 Hot-plate procedure

In this model, the experimental rat or mouse is subjected to an open-ended cylindrical space. The cylindrical space has a floor made of metallic objects which is heated by thermoderm or boiling liquid (Eddy and Leimbach, 1953). Constant heating of the floor metallic object in terms of reaction times results to two behavioural components known as jumping and paw licking. The paw licking behaviours are majorly affected by opioids, in respect to analgesic agents. Conversely, in terms of less potent analgesic agents like acetylsalicylic acid and paracetamol, the time of jumping reaction can be elevated, particularly when the plate temperature is 50 ^{0}C, or when the temperature is increasing in a linear manner, for instance, at 2.5 ^{0}C/min, from temperature of 43 to 52^{0}C (Hunskaar and Hole, 1987). The sensitivity and specificity of the procedure elevated by determining the time of reaction of the initial stimuli irrespective of whether it is jumping or paw-licking or by decreasing the temperature (Milind and Monu, 2013). Relatively stereotyped behaviour is observed in mouse while in rat, the behaviour is more complicated in the rat like licks its hind paws, sniffs, stamps its feet, licks its forepaws, and others. These behavioural manners are regarded as chaotic defensive movements (Knoll et al., 1955)

2.1.4 Pain-state model using cold-stimuli

This model is very rare. It is usually used to determine acute pain and cold allodynia in model of animals with neuropathies (Milind and Monu, 2013). Hence, a new model of animal in respect to determination and stimulation of pain in mice, has been designed and developed. This model designated as M-model, consist mainly of four parts namely, ice floor, ice-tray, Perspex-box and M-zone. Initially, the mouse is subjected to different parts of the M-model basically the M-zone. This is performed for about 60 seconds so that the mouse become sensitive to the M-zone before the commencement of the test (Yadav and Parle, 2016; Saleem and Naz, 2017). The animal is introduced into the model through the top of the Perspex box and the ice block in the ice tray is move to the Perspex box floor. The animal then escape to the M-zone when it could not withstand the cold surface. The time require for the animal to move to the M-zone from the cold ice-floor is known as endurance time and it is recorded with a stopwatch. In general about 4-6 seconds is required for a mouse to escape to the M-zone and avoid the cold ice floor. Narcotic drugs such as pentazocine (10 mg/kg, s.c), tramadol (5 mg/kg, s.c, opioid agonist), butorphanol (2 mg/kg, s.c, partial opioid agonist)and non-narcotic pain-killer drug such as meloxicam (5 mg/kg, sc, preferential COX-2 inhibitor), ketoprofen (5 mg/kg, p.o, non-selective COX inhibitor) and diclofenac (15 mg/kg, i.p, non-selective, COX inhibitor) were pre-administered to groups of animals to evaluate their effect on endurance time. After the administration of standard drugs, the time is observed at 0, 15, 30, 45, 60, 120 and 180 minutes (Yadav and Parle, 2016; Saleem and Naz, 2017).

2.2 Pain state model through mechanical stimuli: mechanical stimulation

2.2.1 Strain gauges

In this method, the punctiform region on the hind paw or not often the tail region received an elevating quantity of pressure. So, the paw or tail of the animal is wedged between a blunt point and a plane surface mounted on top of a cog wheel system. This system has pointer that can be pointed in the length direction of a graduated beam (Green et al., 1951). A step wise reaction takes place when pressure is elevated. Hence, a complicated movement of the experimental animal to free its captured limb or removal of the paw under reflex, followed by vocal response is observed. Randall and Selitto with the target of increasing the procedure sensitivity, gave

comparison of the observed thresholds with the paw with inflammation and with a non-inflamed paw (Randall and Selitto, 1957).

2.2.2 Von-Frey filaments

The assessment of mechanosensitivity is the major procedure for the study of pain models of animal. This method is usually performed by the application of von-Frey filaments in an up-downtesting method. It is the mainly used model for estimating animals' pain as described by Vivancos (Vivancos et al., 2004) for rodents mechanosensitivity testing. But, in this model, the experimental animals are expose to variable quantity of stimuli which may make the animals to move in particular groups and getting wide range of testing exposures that will affect their later response. Therefore, an easy up-down procedure for reckoning paw withdrawal threshold with von-Frey filaments has been developed, in order to standardize the estimation of mechanosensitivity. For a particular test, the method uses five stimuli (Wang et al., 2014).

However, this mechanical stimulation has quite number of demerits. The measurement of stimulus intensity with precision may be difficult to attain. Diminution may be produce as a result of repetition of the process. Also, elevation in the sensitivity of the stimulated part may result to alteration of such part as a result of inflammatory reactions. Thus this can questioned the repeated test validity (Milind and Monu, 2013).

2.3 Pain state model through electrical stimuli: electrical stimulation

Electrical stimulation of the skin can be achieved by applying different types of electrical devices connected to electrodes which stimulate intracutaneous tissue (Brennumet al, 1992) or the skin surface (Curatoloet al, 1998). Stimulation provided by the stimulator devices can be of different types such as different patterns of waveforms, duration and frequency of the stimuli. Different types of nervous structures and afferent nerve fibres are activated as a result of this stimulation. Stimulation may be selective; inducing different types of pain (Woolf et al., 2001; Handwerker et al, 1993). Excitation of nerve fibers results as a consequence of electrical stimulation and the proportion with which these fibers are activated depends on the intensity of the stimulus (Handwerkeret al, 1993). A higher threshold is required for the activation of C-fibers as compared to the A-delta fibers. Studies on different classes of drugs such as opioids, NSAIDs

and tricyclic antidepressants, have been performed using various intensities of electrical stimulation.

Muscles can also be stimulated electrically by the use of fine needles which have un-insulated tips (Laursenet al, 1997). Temporal summation is induced by repeating the electrical stimulation. This can reflect central changes by increasing the areas of referred pain (Laursen et al, 1997) (Schulte et al., 2003). Drugs like remifentanil, alfentanil, morphine, ketamine and oxycodone have been evaluated by the use of this method.To study the basic mechanism of pain as well as the referred pain and the characteristics of pain, gut has been used a lot for the purpose of study and has demonstrated the safety of electrical stimulation in the gastrointestinal tract. To study the neurophysiological pain mechanisms electrical stimulation is considerably more suitable as compared to other methods due to a properly defined offset and onset of the stimulation (Frobert et al., 1994) (Drewes et al., 2003). Electrical stimulation of viscera has been used for the study of drugs such as valdecoxib, morphine, parecoxib, ketamine, and oxycodone.

2.3.1 Electrical stimulation of the tail

The tail of rat or mouse is subjected to subcutaneous electrodes to receive progressively increasing power of electric stimuli which can last for some milliseconds. Application of slow elevation of electric stimuli intensities (for instance, from voltage of 40-50 V), vocalization and impulse motion of the tail take place at the stimulation time. Afterward, the utterance from the animal is observed in the stimulation period and this may result to the death of the animal. In this model, morphine and morphine-like drugs are useful (Laster et al., 1993).

2.3.2 Grid-shock test

In this model, mice with weight between 18-20 g is introduced into a plastic chambers that are clear. The stainless steel wire of the chamber is wired with box floor which has space of about 1 mm apart. Therefore, pulses in the form of square waves, the stimulus is given a period of 2 ms/pulse with 30 cycles/s. when the intensities of the shock is elevated, the mice is observed to gasp, which is an indication of frightening reaction. In addition, effort to jump and increased movement is observed. Pain thresholds of each individual experimental mouse is determined at 15, 30, 60, 90 and 120 minutes before the administration of the testing drugs (Blake et al., 1996).

This pain threshold response is referred to as the behaviour that is correctly directed on the oscilloscope by the marked vacillation.

2.3.3 Stimulation of the tooth pulp

In this model, the experimental animal is subjected to electric current to stimulate the animal's tooth-pulp. Chewing, head flicking, biting and licking are the symbols of pains observed in the animal (Saleem and Naz, 2017). Animal model used in this method are rabbits of both sex and fentanyl-citrate at dose of 0.2 mg/kg and thiopental at dose of 15 mg/kg is administered intravenously to produce anaesthetic effect(Saleem and Naz, 2017). A pulp chamber in lateral margins of the upper incisors is created by a high speed dental drill. The process of kicking will be produced by electric current of 0.2-mA (Steinfels and Cook, 1986).

2.3.4 Monkey-shock titration test

In this model, a monkey is used as the experimental animal and they are kept in restraining chairs. A monkey shock titration is performed and it is the final estimation of a new agent before used in humans. An electric current is passed through a Coulbourn instrument programmable stunner with cathodes linked to two clasps of test tube. The shave part of the experimental animal's tail is connected to the test tube clasps. Afterwards, a current (ranging from 0 – 4 mA) via a 29 progressive steps are passed to the animal. A bar pressed by the experimental monkey suppresses the current used. Stable level of baseline shock is observed for each of the experimental monkey prior to administration of drug such as pentazocine (10 mg/kg, i.m), morphine (3.0 mg/kg, i.m) and methadone (1.7 mg/kg, i.m). But, the Monkey-shock titration method is time consuming (Bloss and Hammond, 1985).

2.3.5 Stimulation of the limbs

In this model, electromyographic recordings of reflexes of nociceptive limbs are used for pharmacological studies of analgesia. However, this model are not common compared to behavioural methods. The electromyographic works will allow reactions determination irrespective of presence of any movement (Saleem and Naz, 2017).

2.3.6 Major disadvantages

With the help of electrical stimulation, no specific nociceptors are activated as it activates the nerves directly, passing the receptors and this is true for both the stimulation of skin as well as muscles and viscera organs. The electrical threshold required to activate a specific nerve fibres is related to that fibre's diameter and it is usually difficult to activate fibres with small diameters as the excitation of the surrounding nerve fibres cannot be avoided (Reddy, et al, 2012). Also, when a specific muscle is stimulated, it twitches which may further confuse the sensation produced by the stimulation of muscles using electrical stimuli (Graven-Nielsen et al., 2001).

2.4 Pain state model through chemical stimuli: Chemical stimulation

Chemical stimulation is achieved through the administration of algogenic chemicals which causes slow, irreversible and progressive stimulation. This model is the closet to clinical pain (Milind and Monu, 2013; Saleem and Naz, 2017)

2.4.1 Formalin

This model is described as a chronic pain procedure, and it is used to determine centrally potent analgesic substances. In this model, after the administration of formalin into the front paw, responses such as excessive biting and licking of the paw are observed. When both paws rest on the floor, it notify protection of the test drug or analgesic response. Scale of four level that is associated with posture is used to evaluate response as painful behaviour. In this scale, the zero level signifies normal position, one signifies that the injected paw stays on the ground, however, not supporting the animal, two signifies that the injected paw is plainly raised, and three signifies that the injected paw is being nibbled, licked or shaken by the animal. Examples of the allogenic agents used in this model includes acetic acid or phenyl quinone (Joshi et al., 2014).

2.4.2 Acetic acid induced writhing procedure

In this model, pain is shown as a characteristic behaviour of abdominal muscles contractions and hind paw stretching together with dorso-abdominal muscles twisting and co-ordination of motor in mice or rats after allogenic agents' administration into the peritoneal cavity causing serous membrane irritation. This process is called the writhing test and it is estimated as per unit of time (Joshi et al., 2014).

2.4.3 Stimulation of hollow organs

In this method, the injection of algogenic agents into hollow organs like colon of experimental animal produce a true visceral pain. For instance, formalin administration into the colon of experimental rat give a multiplex biphasic kind of true pain that causes pain behaviour. This model involves initial stage of contraction of either the whole body or flanks and stretching of the body. The second stage involves the nibbling and licking of the abdomen (Miampamba et al., 1994). Also, abdominal constriction can be produce by glycerol intra-colonic infusion (Botella et al., 1998). Consistently, a quite number of models have been developed for pain in bladder. In this model, administration of capsaicin or turpentine through intra-vesical route has cause multiplex behaviours and/or reflexes (Craft et al., 1993; Jaggar et al., 1998).Another model for pain in inflamed uterus was developed through the intra-uterine injection of mustard oil. This leads to multiplex behavioural patterns in experimental rats (Wesselmann et al., 1998). Probably, a more convincing stimulus of the noxious visceral organ is produced by the hollow organs distension. Inflatable balloon in an experimental rat to produce colo-rectal distension is the mainly used stimulus. Other models involving animals like rabbit can be used for colonic distension (Jensen et al., 1992).

2.4.4 Major disadvantages

Different responses of tested drugs were obtained with the help of the capsaicin model, for example desipramine and lamotrigine which are used to treat the neuropathic pain when tested on this model failed to show any results (Wallace et al, 2002).But in another study, another neuropathicpain relieving drug called gabapentin suppressed hyperalgesia caused by the sensitization of heat-capsaicin (Dirks et al., 2002). Also the use of mustard oil model is limited and it has not been used for testing painkillers or analgesics.Using hypertonic saline injection has disadvantages as they can activate both nociceptive and non-nociceptive nerve fibres. All of the methods of chemical stimulation have problems in reproducibility with large differences inter-individually (Mørket al, 2003). There is a long period of latency before the appearance of effects using the chemical stimulation and this contributes to the major disadvantage of using chemical stimulation to induce pain in pain state models. Also when the chemical stimulation is repeated the responses are not reproducible.

2.5Conclusion

The various models used for inducing pain in different types of animal tissues include the pain-state model which involve the use of chemical, electrical, mechanical and thermal stimuli. However, some of problems including ethics, technics and philosophy are involved in the study of pains using the various models. Understanding the neural mechanism of majority of the models listed is still poor, but, their uses could be vital in envisaging analgesic activity of molecules that are newly discovered.

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