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Animal models of nociceptive pain

Zwierzęce modele nocyceptywnego bólu

INTRODUCTION

Pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". It plays a significant role as an alarm/process that helps to protect the organism and induces learned avoidance behaviors, which may limit the potentially damaging consequences and facilitate fundamental biological functions (e.g. inflammatory or healing) [16]. In contrast to human pain the response to aversive stimuli in animals is referred to as nociception. Moreover, acute pain is a normal defensive function by contrast with chronic pain noted as a pathological state. Progress in pain releasing research has been aided by the development of increasingly sophisticated animal models. Although models of acute nociception in naive animals, such as the tail-flick, hot-plate and writhing tests, were used to develop all of classic analgesic drugs, more recently developed models represent clinically relevant pathological conditions. More importantly, models of inflammatory or neuropathic pain can identify potent and effective drugs that have the potential to be successful in the clinic while leaving animals' in general good health and without excessive distress, weight loss or general behavioral changes.

It is significant that animal models of nociception are considered to be crucial for understanding the mechanism underlying development and maintenance of the pain experience in humans. They allow for an implementation of invasive procedures, experimental studies and manipulation of variables that would be considered unethical if performed on human subjects [12]. Moreover, these experiments using wide-ranging doses of the study drug also allow to obtain preliminary efficacy, toxicity and pharmacokinetic information about a new compound.

Prevalence of pain is still a grave problem; therefore, finding the best animal model and comparison of antinociceptive tests is essential. The main problem in evaluating pain response is that it cannot be monitored directly in animals but can only be estimated by examining responses of animals to nociceptive stimuli which are often not very specific. There is no way to assay the 'quality' of pain in current animal models. A considerable obstacle is the absence of verbal communication with animals [52]. The monitored reactions, are almost always motor responses ranging from spinal

reflexes to complex behaviors. These signs include basic motor responses (withdrawal, jumping), neurovegetative reactions e.g. increase in sympathetic tone (tachycardia, arterial hypertension, hyperpnoea) and vocalization. It can also refer to more complex reactions, which result from the period of learning and sometimes could prevent new damage [47].

In addition, pain studies are importantly affected by a wide range of modulatory factors, including species, strain, age and gender [69], all of which must be taken in to account before the choice of an animal model. The species used in behavioral models of pain are usually rats or mice; however, with the increasing availability of genetically engineered animals, the issues of species or strain differences are of major importance. Moreover methodological factors such as the route of administration, the time of day and the type of the test also have an influence on the observed data [47]. Therefore, it should be noted that there are many tests measuring the antinociceptive effect of substances which vary in stimulus modality (chemical, mechanical or thermal), application site (paw, tail and colon) and intensity of stimulus. In the choice of the test the potency of the substance is taken in to account and whether the response is mediated by spinal reflex or involves supraspinal structures. The kind of the test also depends on what kind of pain is studied. It is especially valid to the proper choice of a prime test which evaluates the analgesic activity of a newly synthesized substances.

Multiplicity of nociceptive tests leads to classification of the kind of stimuli used to induce pain (chemical, thermal, mechanical) or type of evoked behavior as a response to painful stimuli (reflexive behaviors, unlearned or learned behaviors and chronic nociceptive responses). Animal models of pain can be generally classified as either somatic or visceral pain models (such as gastrointestinal pain, urinary bladder dysfunction, etc.). Somatic pain models are the most widely used and include acute nociceptive models (hot-plate, tail-flick) and pathological pain models. Unfortunately none of nociceptive models or used stimuli is ideal.

It should be noted that one of the stimulations in nociceptive animal models is electrical stimulation. This is quantifiable, reproducible and noninvasive stimulus. Apart from this it also has serious disadvantages. First, likewise all the used stimulus, it is not a natural type of impulse, and secondly it excites in a nondifferential fashion all pheripheral fibres (not only nociceptive). The third problem are variations in the impedances of stimulated tissues. Moreover, this type of stimulation could produce problems such as activating the inhibitory mechanisms produced by large diameter, fast-conducting fibers before the arrival of the signal in the finer diameter, slow-conducting fibers [31].

Another stimulus – heat – acts by producing disruption of equilibrium between heating via the arteriovenous capillary bed and heat loss from the skin surface. Skin is stimulated by heat without involving visceral or musculoskeletal tissues. However, heat can activate thermoreceptors then also nociceptors and eventually paradoxical cold receptors. In practice, the animal withdraws itself quickly from the stimulus, and therefore only stimulation of thermoreceptors takes place [34, 37, 47]. Limitation of this stimulation is a weak caloric power of the stimulators and emission radiation which is poorly absorbed and well reflected by skin. Another thermal stimulus are hot water and a CO_2 laser [33]. The advantage of using CO_2 laser is that the absorption of laser heat is independent of pigmentation of the skin or incidence of the radiation, also thermal energy absorbed at the skin surface is concentrated in the region in which the thermosensitive nerves are located because of weak

penetration of this type of heat. Another benefit from this method is elimination of complications of bad contact with skin of animals. Nevertheless, the CO_2 laser is still rarely exploited due to financial and technical reasons. It is essential that thermal tests do not involve visceral or musculoskeletal tissues but stimulate thermoreceptors. Moreover, the source of heat can be distant from its target or can be in direct contact with the skin. But it should be taken into consideration that the application of a thermal stimulus will result in an organized and unalterable sequence of activation, namely thermoreceptors, then thermoreceptors plus nociceptors, then nociceptors alone, and finally (possibly) nociceptors plus "paradoxical cold" receptors [73, 74].

Elicitation of pain in animals may be also caused by mechanical stimulation - nonspecific stimulus activating nociceptors, as well as by mechanoreceptors. Conventional techniques do not allow noxious mechanical stimuli to be delivered rapidly and briefly enough to produce synchronous excitation of the nerve fibers – with disadvantages identical to those discussed above for thermal stimuli. Finally, especially in small animals, such as rodents, the stimulated parts of the body are small, which can produce problems in separating the cause (stimulus) and effect (reaction) [47].

Interesting is the chemical stimulation, which involves the intradermal, intraperitioneal or intraarterial administration of chemical agents and represents a slow, or even very slow, form of stimulation with progressive and inescapable character once they have been applied [24, 79]. These experimental models are the closest in nature to clinical pain.

However, for all kind of tests it is essential that the applied stimulus must not produce lesions, therefore nearly every test has a "cut-off time" (limit for how long the animal should be exposed to the stimulus). This limit is absolutely necessary when the intensity of the stimulus is increasing [10]. However, it should be taken into account that repeated application of a stimulus can sensitize peripheral receptors or produce a central sensitization. These phenomena, in turn, can badly affect the findings during the final phase of the antinociceptive effect [3, 44].

TESTS USING CHEMICAL STIMULUS

These tests involve using an irritant chemical agent as the nociceptive stimulus. The main types of behavioral test on such stimuli use intradermal or intraperitioneal injections. However, intra-arterial injection of bradykinin [23] or intracapsular injections of irritant substances [80] are also used. The most popular test using chemical stimulus administered intradermally is the rat formalin test [18]. In this model pain is induced by acute tissue injury [72] to various species including mice [64], rats, rabbits and guinea-pigs [71]. Pain reaction is observed as flavoring, clutching, elevating, shaking, licking or biting the injected paw [42]. One of the advantages of the formalin test is that it allows producing scaled pain intensities according to the used concentration of formalin solution; however it must be kept as low as possible to minimalize the suffering of the animals [64]. Therefore, the concentration of formalin should be taken into account in planning experiments. Moreover, in this test is observed an early (first 5 minutes after injection) phase of high licking activity or both early and late (20–30 min after injection) phases of reaction after injection of 0.02–0.2% and 1% solution, respectively. It is significant that specific pain behaviors can be captured automatically and therefore the effects of pharmacological substances on motor activity can be identified and uncoupled from antinociceptive effects [42].

Another test using the chemical stimulus is the writhing test. This name comes from a characteristic stretching behavior of animals called writhing. This is a response of animals on intraperitioneal administration of an irritant agent (e.g. 0.02% phenylbenzoquinone solution, 0.6% acetic acid). Antinociceptive activity is expressed as the percentage reduction or inhibition of the number of abdominal writhes [36, 57]. This test detects central and peripheral analgesics activity but also determines activity of some psychoactive agents, e.g. clonidine which could disturb proper results. The advantage of this method is that it allows for the evidence to be obtained for effects produced by weak analgesics. On the other hand, this test lacks specificity because positive (antinociceptive) results are also produced by analgesic noneffective substances (e.g. antihistamines, neuroleptics) [17, 49]. Thus, the positive result with this test does not necessarily mean that the compound has an analgesic activity. A 0.02% phenylbenzoquinone solution given i*ntraperitoneally,* capsaicin or L-glutamic acid injection into the ventral surface of the right hindpaw could also be used as a chemical stimulus to evoke pain reaction. However, the intradermal [39, 46, 65] or i*ntraperitoneal injections* are most frequent routes of stimuli administration. Less common is the use of intraarterial or intradental bradykinin injections [23, 48] and intracapsular injections of chemical substances in nonbehavioral models [80]. In addition, antinociceptive tests may involve injecting irritant substances directly into hollow organs and may be regarded as models of visceral pain [53].

TESTS USING THERMAL STIMULI

There are three main tests using thermal stimuli [34]. The first of them is the hot plate test using a metallic plate heated to a constant temperature of 55 °C [55, 78] to evoke rat pain reaction. Hot plate test produces two basic behavioral components: paw licking and jumping with all four feet. It is interesting that behavioral responses are more complex in rats than in the mice. Rats could react with sniffing, licking its forepaws and hind paws, they straighten up, stamp their feet, start and stop washing itself and others [21]. There are examples of unlearned behaviors more complex than reflexive responses and they appear to represent higher cerebral functioning [12]. It should be taken into consideration that this is a method of evaluating analgesic activity of central origin therefore peripherally acting analgesic show little or even no activity in this test. Moreover, different kinds of reactions are indicated by selected substances, e.g. the paw licking behavior is affected only by opioids, while the jumping reaction time is increased also by less powerful analgesics (paracetamol) [1, 38]. Likewise, this test is very susceptible to learning phenomena, which result in a progressive shortening of the jumping reaction time accompanied by the disappearance of the licking behavior [44]. It triggers shortening the reaction time even by placing the animals on an unheated plate just once a day or once a week and leads to a decrease in the reaction time [61, 66].

The tail-immersion and tail flick are other thermal tests. Tail-immersion described by Janssen et al. (1963) use a water bath heated to 52 °C. The animals' tails are placed in, and the latency of response (in seconds; reflexive withdrawal of the distal half of the tail after its immersion in water) is measured before and after injections of the drugs [40]. The response of the animal depends on the intensity of the heat source. It is the most commonly used method to investigate reflexive

nociceptive behavior in rodents. This is because motor reflexes are inherent in most animals and act as a protective mechanism to avoid tissue damage or injury.

The second test is the tail-flick test, which has two variants. One consists of applying radiant heat to a small surface of the tail which provokes the withdrawal of the tail [69]. The most sufficient advantage of this method is simplicity with small interanimal variability in reaction time. It is significant that the time of the reaction varies with the intensity of the source of radiant heat and the surface of stimulated area [11, 32]. The observed reaction is called a spinal reflex, but it is possible that this is not a purely spinal reflex and also stays under control of supraspinal structures [45, 54]. Moreover this test is prone to a reduction in the response with repetitive stimulation – habituation [27]. The limitation of this test is that it is truly efficient only for revealing the activity of opioid analgesics [28].

In the second variant thermal stimuli involves immersing the tail in water at a predetermined temperature [5, 26, 40]. Although apparently similar, these two alternatives are different at the physical level. The stimulated surface area in tail immersion is far greater than in a tail-flick test. Moreover, the tail immersion test can exploit not only hot but also cold stimuli [60, 77] and it is possible to apply different temperatures to seek evidence for the effects of major and minor analgesics [50, 67].

Thermal tests may involve not only a preeminent organ of thermoregulation in rats and mice – the tail [79]. The stimulus may be applied to the paw of a freely moving animal. It is affordable because the paws of rodents are very sensitive to heat even at temperatures that are not damaging to skin. Moreover, radiant heat may be administrated to a paw that had already been inflamed by a subcutaneous injection of carrageen or exposure to ultraviolet radiation [58]. A disadvantage of this test is that the position of the leg becomes a factor limiting application of this method.

It is significant that models of nociception can also use cold stimuli to evoke pain in animals, including immersion of the tail or a limb [2, 6] or placing the animal on a cold surface [41]. Nevertheless, cold stimuli are rarely used to test acute pain.

TESTS USING MECHANICAL STIMULI

These tests use constant or, more commonly, increasing pressure [75] to the hind paw or the tail [25]. The measured parameter is the threshold (weight in grams) for the appearance of a given behavior. When the pressure increases the reflex withdrawal of the paw can be observed, release of the trapped limb or a vocal reaction. This kind of stimulation have many disadvantages, e.g. to measure the intensity of the stimulus with precision is difficult, repetition of the stimulus can produce diminution or sensitization of the stimulated part of body, relatively high pressure must be applied and finally a relatively small number of substances are active in this test [47]. Moreover, to improve the sensitivity of the test a comparison of results observed for a healthy and inflamed paw is used [63].

TESTS USING ELECTRICAL STIMULI

Test using the electrical impulse is an example of learned behavior. Animals must at first learn to avoid or discontinue the noxious stimuli, such as a controlled electrical shock, by releasing a lever. The most important weakness of this stimulation lies in the nonselective way in which it excites

different types of nerves; moreover, application of this stimulus is difficult. It is used to activate pain reaction of the tail, dental pulp or limbs [15]. As responses for this stimulation the following are recorded: animal twitching, squeaking, and attempting to escape by jumping, or vocal responses [20].

The sensation of dental pulp is the most commonly used to estimate pain reaction because of its selectivity. This statement was based on three major facts: firstly – it was indicated that afferent nerves in the pulp in humans can be activated only by pain-producing stimuli, secondly – activation of these nerves in humans produces only pain sensation, and thirdly these fibres have small diameters with slow conduction velocities like those associated with nociception in other parts of body [76]. The selectivity of the test is true at least for teeth of limited growth such as those in the dog and the cat [7, 9, 43, 51]. Some unsolved problems are with continuously erupting teeth. They are anatomically different, and some previous studies have shown that the application of electrical stimuli could excite not only the pulpal fibres but also the nerves outside the tooth (periodontal nerves) [19, 35]. Therefore, it was concluded that there are reasons for caution before ascribing all the responses to electrical stimulation of rodent incisors as being nociceptive. Despite these problems, many species with continuously erupting teeth (rabbits, guinea pigs, rat) have been used in this test in addition to species with the teeth of limited growth [19, 35, 70]. As a response to painful stimuli coordinated reactions have been observed like licking, chewing, changes of facial expression, and head movements in the awoken animal [29, 59, 62, 68]. Some observations showed that such reflexes were produced by stimulation of mechanoreceptors [8, 30] as well as by stimulation of nociceptors. Despite the limitations of this models, they discriminate well opioid analgesics [13, 14, 22] and can reveal the activity of nonopioid analgesics but not nonsteroidal anti-inflammatory agents.

The electrical stimulation of the limbs is less common. This is caused by the variety of preparations which should be used [4, 56].

CONCLUSIONS

No test of nociception meets all required criteria so the choice of a test has to be a compromise. Ideally, a behavioral model for nociception in an animal should possess a few characteristics specified below.

Firstly, the choice of nociceptive predictiveness is essential in nociceptive tests. It is necessary to avoid false results in searching for new molecules with therapeutic value [13, 14]. For example, the writhing test is very sensitive but weakly predictive and therefore has to be reserved for initial pharmacodynamic screening so that potentially analgesic substances are not missed [17, 49]. From nociceptive tests the most predictive of the models of acute pain are the formalin test [18], the tail-flick and hot plate tests. Nevertheless, these tests are active only for substances which possess the mechanism of action similar to that of morphine [13].

Next, the model of antinociception must be sensitive to manipulations and show effects for different classes of antinociceptive agents at doses comparable to those used for analgesics in humans. Moreover, they must allow the differentiation of nonspecific behavioral changes from those triggered by the nociceptive stimulus itself. It is significant that repeated application of the stimulus must not produce lesions and the results obtained with a test must be reproducible.

It should also be taken into account that using behavioral tests involves subjective observation for several minutes. This makes the tests tedious, time-consuming and experimenter-dependent. Therefore, automate behavioral tests could allow researchers to use more sophisticated and more predictive tests than the usual acute pain tests [42].

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SUMMARY

Pain is one of the most common symptoms experienced by both humans and animals. In humans, unrelieved pain can disturb daily activities and quality of life. Animal models should provide basis for a better understanding of the pain pathomechanisms in humans and give information on the therapeutic value of novel drugs. The main problem of using animal models is that pain cannot be monitored directly but only estimated by examining animal responses to nociceptive stimuli. The animal reactions to nociceptive stimuli are almost always autonomic or motor responses ranging from spinal reflexes (tail withdrawal) to such behaviors as licking, jumping, writhing or vocalization. However, such responses do not necessarily mean that animals can distinguish a wide variety of painful sensations. There are many different types of nociceptive stimuli (electrical, thermal, mechanical, or chemical) that are used in different pain models. Animal models of pain should have features of clinical pain and reproducibility. Nevertheless, none is ideal, although chemical stimuli probably most closely mimic acute clinical pain. Models of nociception that can be reproduced in different species are of the greatest value to drug development, as protein or receptor heterogeneity between different species is always possible. However, complex physiological experience of pain cannot be completely evaluated by using only animal subjects in a laboratory setting.

Keywords: pain, nociception, nociceptive stimuli, nociceptive tests, animals

STRESZCZENIE

Ból jest wspólnym symptomem doświadczanym przez ludzi i zwierzęta. U ludzi nieustający ból może zburzyć aktywność życiową i obniżyć jakość życia. Zwierzęce modele bólu powinny służyć do lepszego zrozumienia patomechanizmu bólu i dostarczać informacji o terapeutycznej wartości nowych leków. Głównym problemem w używaniu tych modeli jest to, że ból nie może być bezpośrednio monitorowany u zwierząt, ale jedynie oszacowany przez obserwację reakcji na bodziec nocyceptywny. Zachowania obserwowane u zwierząt są zwykle autonomiczną czy motoryczną reakcją na bodziec bólowy, ocenianą jak odruch rdzeniowy („wyrzucanie" ogona) czy inne zachowania, jak lizanie łap, wyskok, przeciąganie tułowia, wokalizacja. Jednak takie odpowiedzi niekoniecznie oznaczają odczuwanie różnorodnych wrażeń bólowych. Jest wiele różnych typów bodźców nocyceptywnych (elektryczne, termiczne, mechaniczne czy chemiczne) używanych w modelach zwierzęcych. Zwierzęcy model bólu powinien posiadać cechy bólu klinicznego i być powtarzalny. Jednak żaden z modeli nie jest idealny, chociaż bodziec chemiczny najbardziej naśladuje ostry ból kliniczny. Takie modele bólu, które mogą być odtwarzane u różnych gatunków, mają największe znaczenie farmakologiczne dla poszukiwania nowych leków, chociaż różnice gatunkowe są zawsze możliwe ze względu np. na heterogeniczność receptorową u zwierząt. Jednakże skomplikowany psychologiczny aspekt odczuwania bólu nie może być oceniony przy użyciu jedynie modeli zwierzęcych.

Słowa kluczowe: ból, nocycepcja, bodźce nocyceptywne, zwierzęta