Analgesia in phasic and tonic pain tests in a pharmacological model of autotomy

Suman Jain & Ratna Sharma*

Department of Physiology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110 029, India

Received 22 May 2002; revised 21 August 2002

Self-mutilation or self-injurious behaviour is a well known behavioural disorder in humans. The proposition that this behaviour in animals is a response to chronic pain of peripheral nerve injury has been met with controversy. In the present study a pharmacological model, which produces no sensory or motor loss was used to study how autotomy is related to pain. In a group of rats autotomy was induced by amphetamine in phenoxybenzamine and reserpine treated animals. The pain tests, both phasic and tonic were then performed. The results of this study showed that a total analgesia was produced in both phasic and tonic pain tests, in animals that exhibited autotomy. Injection of naloxone in these animals prevented autotomy. A correlation between autotomy and no pain is suggested in this pharmacological model of autotomy.

Chronic neuropathic pain syndromes, pain associated with injury or disease affecting the nervous system, are among the most intractable conditions encountered in clinical practice. Researchers interested in this problem have long realised the importance of establishing animal models of neuropathic conditions. Such models would provide not only an area for examining the physiological mechanisms involved, but would also permit testing of new treatment ideas. One such model was based on the observations that after major denervation of a limb, some animals begin to lick and bite the limb, eventually injuring one or more toes. This behavioural peculiarity was termed as 'autotomy'. Further it was proposed that it results from unpleasant and perhaps painful paresthesias referred to the denervated limb. Humans frequently experience intractable pain following either peripheral or central neuronal injury. Thus autotomy has been proposed as a model of chronic pain. However, whether autotomy behaviour is a response to the pain or the chronic paresthesia of peripheral nerve injury, or to some other cause, remains debatable. Obviously if this issue could be resolved, then a better understanding of the neurophysiology of this behaviour would help elucidate the mechanisms of the human deafferentation pain conditions.

Autotomy or self-mutilation can also be induced in animals by altering the neurotransmitter levels by using drugs like amphetamine, which is a catecholamine-releasing agent. Initially stereotyped behaviour manifests itself as sniffing, which when the stereotypy is more intense, changes to licking or biting, which may progress to self-biting. It has been shown by several experiments that the transition from sniffing to licking or biting can be influenced by removal of non-dopaminergic, probably noradrenergic mechanisms.

We have reported earlier, that autotomy could be produced in rats by injecting reserpine or phenoxybenzamine before administration of amphetamine. The present study was conducted to find out how autotomy is related to pain and what is the involvement of opioidergic drugs in this behaviour.

Animals—Adult male wistar rats weighing between 200 to 300 g, were used for the study. They were housed in an animal room having controlled room temperature (26°±2°C) and lights on from 05:00 to 019:00 hrs. Food and water were available to them adlibitum. Ethical guidelines were followed in accordance with the committee for research and ethical issues of IASP and it was approved by the ethical committee of All India Institute of Medical Sciences, for conducting animal research.

Behavioural tests
(1a) Stereotyped behaviour—Stereotyped behaviour was scored by the scoring system described by Creese and Iverson. The salient features of this system are as follows: 0: asleep, 1: active, 2: predominantly active with bursts of stereotyped sniffing or rearing, 3: stereotyped activity along a fixed path, 4: stereotyped sniffing or rearing maintained in one location, 5: stereotyped behaviour in one
location with bursts of gnawing or licking, 6: continual gnawing or licking of the cage.

(1b) Autotomy—The salient features of the scoring system for self-biting behaviour are as follows: 0: no biting, 1: occasional biting, 2: frequent biting and keeping the paw for a longer duration in mouth, 3: fast and forceful biting with bleeding from tissue, 4: frequent biting with removal of one or more phalanges.

(2) Pain tests

(a) Phasic pain tests

(i) Tail flick test—Each rat was conditioned in the restrainer for 30 min. The tail was cleaned with spirit. Radiant heat (45° ± 2°C) was focused onto the ventral surface of the tail (approx. 5 cm from the caudal end of the tail) and the tail flick latency (TFL) was noted using the Tail Flick Analgesia Monitor (Omnitech, USA). The procedure was repeated thrice at an interval of 5 min. The cut off time was set at 30 sec to avoid tissue damage. The mean of three observations was taken as the basal TFL.

(ii) Hot plate test—Each rat, one at a time, was placed on the hot plate, whose temperature was maintained at 52.5°C. The time the rat takes to start licking its hindpaw from the entry into the hot plate chamber was recorded as the hind paw lick latency (HPLL) in sec. The cut off time was set at 40 sec to avoid tissue damage. The procedure was repeated thrice at an interval of 5 min. Test value was the average of three readings.

(b) Tonic pain test

Formalin test—Each rat was conditioned in the restrainer for 30 min. They were then injected (sc) with 5% sterile formalin solution (50 μl) into the planter surface of the forepaw. The pain related behaviour was quantified by using a four-point scale as described by Dubusson and Dennis. The salient features of this scale are as follows: 0 (when the whole body is resting or the rat is moving on all forepaws), 1 (grooming or the injected paw was partially resting on the floor), 2 (The injected paw was kept elevated or tucked into the body), 3 (The injected paw was licked or shaken). The behavioural scores were fed into the computer for 1 hr and the time spent in each category T0, T1, T2 and T3 were continuously recorded in seconds. The average pain rating for each 5 min epoch during one hour of observation was obtained.

The phasic pain tests were done in random sequence in different rats so as to rule out the effect of one test on another. The formalin test, however, was done after the phasic pain tests to rule out the effect of tonic pain on these tests.

Experimental Design

Pain tests (both phasic and tonic) were done in all the rats before any drug treatment. The animals were then assigned to any of the following groups. Following drug treatments, pain tests, stereotyped and autotomy behaviour was assessed in all the animals.

Group I Amphetamine was injected in the dose of 5 mg/kg ip in 10 rats.

Group II Phenoxybenzamine was injected in the dose of 5 mg/kg, ip, 1 hr before injecting amphetamine (5 mg/kg, ip) in 10 rats.

Group III In eighteen rats, 7.5 mg/kg reserpine was given intraperitoneally. 24 hr prior to 5 mg/kg (ip) phenoxybenzamine treatment. Thirty minutes after phenoxybenzamine injection 5mg/kg (ip) amphetamine was injected.

Group IV Naloxone (5mg/kg, ip) was injected in seven reserpinized and phenoxybenzamine treated rats, 20 min before amphetamine injection.

Analysis of data

Kruskal Wallis test was applied initially to find out the variation amongst the different groups. Then to find out the level of significant difference within the groups Multiple Range test was used.

Results

(1a) Stereotyped behaviour—In eight rats of group I and in nine rats of group II, stereotyped behaviour was observed, which corresponded to a score of 3-5. In these rats initially there was an increased locomotor activity or sniffing which developed into severe sniffing or licking of one area. Some of the rats also showed continuous circular movements or fast to and fro head movements. The pain tests were done 1-2 hr after amphetamine injection.

(1b) Autotomy behaviour—The injection of reserpine and phenoxybenzamine before amphetamine injection induced autotomy in twelve rats, which corresponded to a score of 2-3. The autotomy was observed in these rats approximately 1 hr after injection of amphetamine. Initially the rats manifested stereotyped behaviour, during which they preferentially licked
the paw/tail that was later bitten. The biting behaviour was not specific to any of the paws, but once a paw or tail was chosen, the animal continued to bite it. No autotomy was observed in animals of the groups I, II and IV. The pain tests were done while the animal was biting or after 1-2 hr of amphetamine injection.

(2) Pain tests

(a) Phasic pain tests

(i) Tail flick test—The basal TFL ranged from 9.74 to 21.13 sec, with a mean of 14.87±1.38 sec (Fig. 1). Following induction of autotomy (group III), all the twelve rats did not flick their tail till the cut off time of 30 sec, indicating complete analgesia. Naloxone injection in group IV rats, decreased the TFL significantly ($P<0.001$) to 24.39±8.86 sec, ranging from 10.36 sec to 30 sec. In the group I and II rats that did not show autotomy, the TFL ranged from 24.67 to 30 sec, with a mean of 29.2±0.76 and 11.73 sec to 30 sec with a mean of 22.87±2.68 sec, respectively. In group I seven out of eight and in group II four out of nine rats, which showed stereotypy, did not flick their tail till the cut off time. However, when compared with the basal data, the TFL was significantly ($P<0.001$) higher in all the groups.

(ii) Hot plate test—The basal HPLL ranged from 6.38 to 26.82 sec, with a mean of 14.77±2.00 sec (Fig. 1). A significant ($P<0.001$) increase in HPLL was observed in the rats in whom autotomy was observed, when compared to the basal. Ten out of twelve rats did not lick their hindpaw till the cut off time of 40 sec. The HPLL ranged from 23.6 to 40 sec, with a mean of 38.5±4.94 sec. Following naloxone injection, the HPLL decreased significantly ($P<0.01$) to 29.19±13.82 sec, ranging from 5.95 to 40 sec. Four out of seven rats in group II, which did not show autotomy also did not lick their hindpaw till the cut off time. The HPLL following phenoxybenzamine pretreatment ranged from 19.85 to 40 sec, with a mean of 31.97±3.79 sec. However, all the rats of group I, which showed stereotyped behaviour, did not lick their hindpaw till the cut off time. There was a significant ($P<0.01$) increase in the HPLL of all the groups when it was compared to the basal.

(b) Tonic pain test

Formalin test—The self biting behaviour as well as the stereotyped behaviour observed in the rats were so intense that when they were kept in the formalin chamber for conditioning and while testing also, most of them either kept biting themselves or kept sniffing/licking into one of the holes present in the rear wall of the chamber.

The basal average pain rating ranged from 1.77 to 1.98 with a mean of 1.9±0.02. In rats manifesting autotomy (group III), no response to formalin injection was observed. They did not show any discomfort during or after formalin injection. The average pain rating in these rats ranged from 0.0 to 0.92, with a mean of 0.12±0.28 (Fig. 1). In eight out of twelve rats, the pain rating was zero. Following naloxone injection also not much response to the formalin injection was observed, except for one rat, whose average pain rating for one hour was 1.67. The average pain rating in the naloxone injected rats was 0.31±0.66. However, in group II rats in whom no autotomy was observed, a significant ($P<0.001$) in-
crease in the average pain rating was observed, when compared to groups I, III and IV. It ranged from 0.03 to 1.77, with a mean of 1.05 ± 0.25. In group I rats, not much response to formalin injection was observed. The average pain rating in these rats ranged from 0.0 to 0.64, with a mean of 0.23 ± 0.1. In all the groups a significant (P<0.001) decrease in the average pain rating was observed, when it was compared to basal.

Discussion

In the present study blockage of norepinephrine action in amphetamine induced stereotyped behaviour produced autotomy in 66.6% of animals as compared to no autotomy in animals treated with amphetamine only. This is in line with earlier studies\(^9,14\) which show the change of stereotyped sniffing in rats to self biting when pretreated with reserpine, phenoxylbenzamine, diethylidithiocarbamate or 6-hydroxydopamine. It has been suggested that norepinephrine (NE) has inhibitory influences on amphetamine-induced stereotypy\(^14,15\) and removal of noradrenergic influences would produce stronger stereotypy which is manifested as biting behaviour.\(^5\)

The role of NE in deafferentation model of autotomy is controversial. In this model, decrease in spinal monoamine levels has been shown to coincide with maximum intensity of autotomy\(^16-17\). Coderre and Melzack\(^18\) showed that lesion of locus coeruleus produced an increase in autotomy. Intrathecal administration of clonidine\(^19-20\) and medetomidine\(^21\) also suppresses autotomy induced by sciatic nerve lesions. A delay in the onset of autotomy in rats injected with 6-OHDA (which produced a fall in NE) delayed the onset of autotomy when injected 2 days prior to nerve section\(^22\). These and other similar studies do suggest a lack of clear understanding of the role of NE in modulating deafferentation autotomy. In the present study and in an earlier study from our lab, however, both phenoxylbenzamine and reserpine were able to induce autotomy in amphetamine-induced stereotypy. The question that remains to be answered, however, is what causes autotomy, is it pain or lack of it?

Although autotomy is suggested to be a model of chronic pain, what happens to the perception to various kinds of pain sensations have not been studied. This is because in the deafferentation model of autotomy it is not possible to evoke any motor response to a painful stimulation, which is generally used to assess pain in animals. The present study gave a unique situation to study the controversy in literature. As no sensory/motor loss was there in this pharmacological model, the measurements of the changes in the pain thresholds were tested in both phasic and tonic pain tests. Pain tests both phasic and tonic showed a complete or nearly complete analgesia in animals showing autotomy. Injection of naloxone produced no autotomy in any of the animals and the pain ratings in these animals showed partial analgesia. Animals showing stereotyped behaviour only (before NE blockade) also showed lower pain ratings as compared to control pain ratings. The analgesic role of amphetamine is well known and it has been shown to be effective analgesic in humans\(^23,24\) and animal models\(^25-27\). In the absence of NE action this analgesia is potentiated, signifying the role of dopamine in amphetamine induced analgesia. Further it has been demonstrated that substances which block alpha 1 receptors (e.g. prazosin) result in analgesia and substances that block alpha 2 receptors block analgesia\(^28\). This explains analgesia in the animals manifesting autotomy with reserpine (NE depleter) and phenoxylbenzamine (mainly alpha 1 receptor blocker) pretreatment, in the present study. These observations also suggest very clearly that pain rating remain low in animals manifesting autotomy. If any correlation between autotomy and pain is to be concluded then autotomy was produced in animals with no pain.

Further naloxone does not block analgesia produced by stimulation of alpha 2 receptors. In the present study, therefore, in the absence or low levels of NE, naloxone was probably not able to block the analgesia produced by the endogenous system, as shown by the pain tests. Analgesia produced in these animals, however, was less than that produced in animals showing autotomy. A decrease in autotomy with opioid blockers has been reported earlier\(^29,30\). In humans also, it has been shown\(^31-33\) that naloxone attenuates self-injurious behaviour (SIB). This behaviour has been shown to be related to both pain and no pain in Borderline personality disorder (BPD). BPD-no pain patients report feeling less pain and enhancement in mood during an experimental pain procedure\(^34\), which suggests that the endogenous pain system is activated in these patients. Naloxone treatment in these patients decreased self-injurious behaviour\(^32\), as observed in our animals also.

The presence of hyperalgesic state during autotomy as proposed earlier\(^31,35\) cannot be ruled out, as a comparable situation occurs in BPD-pain patients who report increased pain during SIB. Coderre et al.\(^31,36\) have reported that prior injury in the neurac-
tomy model enhances autotomy. Their results strongly suggest that the autotomy is due to a sensory phenomenon, which, in terms of human experience would be, described as pain or dysesthesia. In the present study, however, autotomy was not due to altered sensory experience in the periphery as in the deafferentation model. The pharmacological manipulation of neurotransmitters in the brain produced altered central state of pain perception, which resulted in peripheral analgesia as measured by both phasic and tonic pain tests. In the deafferentation model also, 12, lesion of locus coeruleus enhanced autotomy and the authors concluded that the autotomy is subject to tonic inhibition due to descending controls from the locus coeruleus. In another paper by the same authors, however, systemic injection of NE and monoamine oxidase inhibitor enhanced autotomy. In both these papers the role of NE has been suggested although the conclusion is that autotomy is produced by pain and procedures that augment pain augment autotomy.

However, the results of the present study suggest that autotomy could be due to altered level of the neurotransmitters in the brain i.e. a decrease in norepinephrine along with an activation of the endogenous analgesic system, resulting in no pain condition. Reports from the literature suggest that altered level of neurotransmitters possibly in the striatum, substantia nigra, ventral tegmental area and limbic area are involved in the processing of nociceptive information and also influence the autotomy behaviour.

Acknowledgement

The financial support provided by Institute Research Grant from All India Institute of Medical Sciences, New Delhi, India is acknowledged. Thanks are also due to Mr Sadhu Ram and Mr Sanjeev for technical assistance.

References


18 Codere T J & Melzack R, Procedures which increase acute pain sensitivity also increase autotomy. *Exp Neurol*, 92 (1986b) 713.


