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# Serotonin-Induced Gnawing in Rats: Comparison with Tail Pinch-Induced Gnawing

ALICE A LARSON AND MARIE H KONDZIELSKI

*Department of Veterinary Biology, 295 Animal Science/Veterinary Medicine*
*University of Minnesota, St Paul, MN 55108*

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LARSON, A A AND M H KONDZIELSKI *Serotonin-induced gnawing in rats Comparison with tail pinch-induced gnawing* PHARMAC BIOCHEM BEHAV **16**(3) 407–409, 1982 —The intrathecal (IT) injection of serotonin in the rat was found to produce a variety of behavioral signs of the serotonin "syndrome" as well as intense gnawing Serotonin-induced gnawing is qualitatively similar to that evoked by tail pinch Paradoxically, tail pinch-induced gnawing has been previously shown to be inhibited by serotonergic activity while we found a positive dose-related correlation between the IT administration of serotonin and gnawing Pretreatment with methysergide IT completely blocked gnawing produced by intrathecally injected serotonin but not that evoked by tail pinch In contrast, naloxone, reported to inhibit tail pinch-induced hyperphagia, failed to affect serotonin-induced gnawing The parenteral injection of haloperidol inhibited both tail pinch-induced and serotonin-induced gnawing Failure of intrathecally injected haloperidol to inhibit serotonin-induced gnawing indicates that dopamine mediates this behavior at a supraspinal level The ability of serotonin to elicit gnawing when injected at the spinal cord level, but to inhibit the same behavior when evoked by tail pinch, suggest that this neurotransmitter plays opposite roles depending on which part of the CNS is involved

Gnawing Tail pinch Serotonin Dopamine Intrathecal injections

IT has been established that tail pinch in the rat induces gnawing and eating behavior The pressure applied typically does not evoke vocalization and was originally described as mild [3,4] However, this gnawing behavior was subsequently found to be inhibited by naloxone, and therefore postulated to involve the release of endogenous opioids [10] This would suggest that a certain degree of pain or stress is involved in producing this behavior The gnawing behavior has also been shown to be inhibited by haloperidol and pimozide [3,4] and therefore thought to be mediated by dopamine (DA) Tail pinch-induced gnawing has been shown to be inhibited by serotonergic activity evoked by fenfluramine [1, 2, 11] In contrast, we have observed qualitatively similar gnawing behavior by the intrathecal (IT) administration of serotonin in rats

The present study was designed to characterize this serotonin-induced gnawing in rats In addition, we examined the role of serotonin in tail pinch-induced gnawing as well as the involvement of DA in serotonin-induced gnawing Intrathecal administration of drugs, using permanently indwelling cannulas, allowed us to distinguish the spinal from supraspinal actions of these neurotransmitters The possibility that tail pinch-induced gnawing involves activation of descending serotonergic pathways was studied using IT injections of methysergide The involvement of a dopaminergic synapse in serotonin-induced gnawing was similarly investigated using intraperitoneal (IP) or IT injections of haloperidol

## METHOD

Sprague-Dawley albino rats weighing between 350 and 400 g were used in this study Animals were chronically implanted with intrathecal cannulas (constructed of PE10 tubing inserted 7 cm) according to the method described by Yaksh and Rudy [13] An injection of saline was made to clear the catheter of any debris accumulated during insertion Rats were anesthetized with ether during cannulation and then allowed a minimum of 7 days to recover During the recovery period, rats were given food and water ad lib Prior to the experimental procedure, all rats were allowed to adapt to short periods in plexiglass restrainers and familiarized with the experimental procedure Injections of water IT in a volume equal to the maximum used for any given trial resulted in gnawing in only 3 6% of the rats None of the rats exhibited spontaneous gnawing at the time of testing A minimum of one week separated drug injections for any given animal

Serotonin oxalate (266 3 g/mol) was purchased from Sigma Chemical Co (St Louis, MO) Serotonin solutions were made fresh daily by dissolving the compound in water, making concentrations such that the maximum volume injected never exceeded 20 $\mu$l Serotonin solutions were kept on ice until immediately before use Haloperidol (McNeil Co , Ft Washington, PA) was a gift from Dr A E Takemori of the University of Minnesota (Minneapolis, MN) The compound was dissolved in 0 01 N hydrochloric acid Haloperidol was administered in a 10 $\mu$l volume when injected IT and a 0 1 ml/100 g volume for IP injections Methysergide maleate, a gift from Sandoz Pharmaceuticals (East Hanover, NJ), was dissolved in water an injected IT in a volume of 10 $\mu$l Naloxone hydrochloride was a gift from Endo Laboratories, Inc (Garden City, NY) All drugs injected IT were followed by a flush with 10 $\mu$l of water

TABLE 1
PERCENT OF RATS GNAWING*

| Pretreatment | After Tail-Pinch | After 160 μg Serotonin IT |
|---|---|---|
| Sterile Water | 74 1 | 55 6 |
| Methysergide† (50 μg/rat IT) | 100 0 | 0 |
| Haloperidol‡ (2 5 mg/kg IP) | 44 4 | 11 1 |
| Naloxone§ (5 mg/kg SC) | — | 44 4 |

*n=9 for each group except control Tail-Pinch where n=27
†5 min before testing
‡4 hr before testing
§15 min before testing

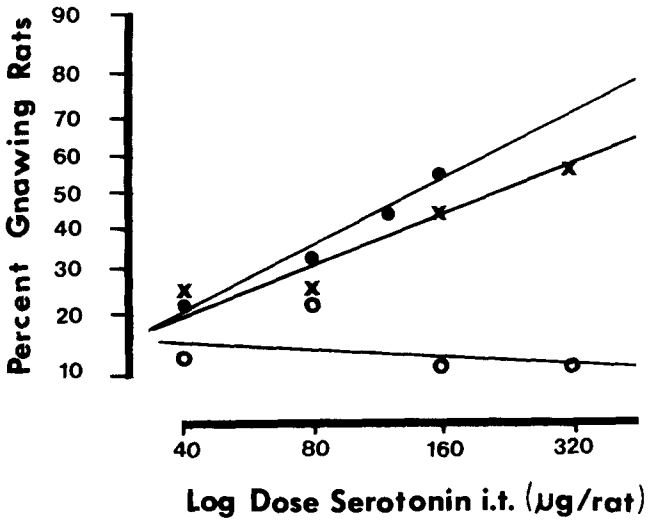


FIG 1 Effect of haloperidol on serotonin-induced gnawing in rats The control $ED_{50}$ obtained for serotonin (closed circles) did not differ significantly from that obtained 15 min after the IT administration of 10 μg/rat of haloperidol (X) However the IP injection of 2 5 mg/kg of haloperidol 4 hr before testing with serotonin, inhibited gnawing (open circles) Each point represents the value obtained from a group of at least 8 rats

Gnawing was recorded as an all or none response and arbitrarily defined as any chewing on the animal restrainer or on a wooden stick placed horizontally in the restrainer (prior to the injection of any drugs) within 5 min of an IT injection of serotonin or tail pinch The tail pinch simply employed a plastic hemostat (MacBick Co generously supplied J E Morley and A S Levine, VA Medical Center, Minneapolis, MN) slowly applied 3 in from the base of the tail Rats were not touched or manipulated until removal of the hemostat

Data was expressed simply as the precent of animals that gnawed Where indicated, dose-response curves for serotonin, injected IT, were constructed The $ED_{50}$ values and their confidence limits and the significance of the potency ratio between two $ED_{50}$ values were determined by the method of Litchfield and Wilcoxon [9] A minimum of 8 animals were used at each dose and four doses tested for each dose-response curve

## RESULTS

Intrathecal administration of serotonin into the lumbosacral area of the spinal cord produced intense gnawing in rats (Fig 1) A variety of other responses which are typically associated with serotonergic activity, such as tremor, rigidity, hindlimb abduction and straub tail, were also frequently observed Gnawing usually began shortly after the IT injection of serotonin and continued for less than 5 min When quantified as an all-or-none response, serotonin produced a dose-related increase in the percent of rats exhibiting this gnawing behavior

The IT administration of methysergide at a dose of 50 μg/rat 5 min prior to the IT injection of 160 μg of serotonin completely blocked the serotonin-induced gnawing In contrast, this same pretreatment with methysergide not only failed to inhibit tail pinch-induced gnawing, but actually increased the percent of rats responding to tail pinch (Table 1)

Naloxone also appears to produce differential effects on the gnawing behaviors elicited by either tail pinch or serotonin Pretreatment with 5 mg/kg of naloxone SC did not appear to affect the percent of rats which gnawed in response to 160 mg of serotonin to injected intrathecally (Table 1)

An IP injection of 2 5 mg/kg of haloperidol 4 hr prior to testing decreased both tail pinch- as well as serotonin-induced gnawing (Table 1) However pretreatment with 10 μg of haloperidol, injected directly into the spinal cord area via the intrathecal cannula, did not inhibit the gnawing behavior produced 15 min later by intrathecally injected serotonin (Fig 1)

## DISCUSSION

Intrathecal adminstration of serotonin causes analgesia [14, 12, 8] as well as several behavioral characteristics of the "serotonin syndrome" hindlimb abduction, tremor, straub tail and limb and axial rigidity [7] In addition to these effects, the present study has demonstrated that the intrathecal administration of serotonin produces gnawing in a dose-related fashion Behaviorally, this serotonin-induced gnawing also appears to be indistinguishable from that produced by tail pinch

Both serotonin-induced and tail pinch-induced gnawing involve a dopamine synapse, as shown by the decreased incidence of gnawing after the IP injection of haloperidol This is in agreement with several previous reports concerning tail pinch-induced gnawing and hyperphagia [3,4] However the IT administration of haloperidol did not decrease serotonin-induced gnawing This suggests the involvement of dopaminergic synapses located specifically in the supraspinal area, and also rules out the possibility of a simple antagonism of spinal serotonergic receptors by haloperidol

The serotonin-antagonist methysergide, injected intrathecally, completely blocked the gnawing elicited by the IT injection of serotonin This indicates that serotonin receptors in the spinal regions are involved in the initiation of this behavior Intrathecally injected methysergide did not block tail pinch-induced gnawing, and may even have

slightly potentiated it (Table 1) Therefore, in contrast to serotonin-induced gnawing, tail pinch-induced gnawing does not depend on stimulation of spinal serotonergic receptors

A second notable difference between tail pinch-induced gnawing and serotonin-induced gnawing also lies in their manner of initiation The IT injection of serotonin has been shown to produce analgesia [8,14], and the gnawing elicited does not appear to be inhibited by naloxone (Table 1) On the other hand, tail pinch-induced hyperphagia is inhibited by prior treatment with naloxone [10], and is thought to involve a degree of pain or stress which causes the release of endogenous opiates

Tail pinch-induced hyperphagia has been shown to be completely suppressed by the serotonergic anorectic agent fenfluramine [2,11] A variety of other manipulations, such as precursor loading and inhibition of serotonin reuptake, which also result in an increased serotonergic activity in the CNS, have also been shown to inhibit gnawing behavior [1] In light of our results, showing the production of gnawing behavior by the injection of serotonin into the spinal cord area, this neurotransmitter appears to have two different and opposite actions one supraspinally and the other at the spinal cord level An enhancement of serotonergic activity at the supraspinal level has been shown to inhibit tail pinch-induced gnawing by inhibiting the release of dopamine in the caudate nucleus [5] The concept of a dual action of serotonin is in agreement with Davis *et al* [6] who showed two opposite effects of serotonin on the acoustic startle reflex, depending on which level of the CNS was injected

In summary, both the intrathecal injection of serotonin and a mechanical tail pinch will produce similar gnawing behaviors, which appear, in both cases, to be mediated by supraspinal dopaminergic synapses Serotonin-induced gnawing requires spinal serotonergic activation, also known to cause analgesia However tail pinch-induced gnawing does not depend on a spinal serotonergic synapse and may actually be pain or stress related

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