Pharmacology Biochemistry & Behavior, Vol. 40, pp. 301–304. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

The Effect of Tryptophan Supplementation on Autotomy Induced by Nerve Lesions in Rats

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Received 11 June 1991

ABBOTT, F. V. AND S. N. YOUNG. *The effect of tryptophan supplementation on autotomy induced by nerve lesions in rats.* PHARMACOL BIOCHEM BEHAV **40**(2) 301–304, 1991.—Rats were fed an artificial diet containing either their normal, or five times their normal, daily requirement of tryptophan for up to five weeks and were tested in an animal model of deafferentation pain, nerve lesion-induced autotomy. In this model one of the hind limbs of the animal was denervated, and the extent to which the animal attacked its denervated paw was assessed. Rats receiving the high-tryptophan diet showed significantly lower levels of autotomy, compared to rats fed the control diet. 5-Hydroxytryptamine and 5-hydroxyindoleacetic acid in the brain and spinal cord were significantly elevated in rats receiving the high-tryptophan diet, indicating that the supplemented diet produced a chronic increase in CNS indoleamine metabolism. Currently there is no accepted pharmacological treatment of deafferentiation pain. Our results suggest that tryptophan should be tested in phantom limb pain and other deafferentation pain syndromes.

Tryptophan Autotomy Deafferentation pain Peripheral nerve injury Phantom limb pain 5-Hydroxytryptamine

ANIMAL studies indicate that 5HT modulates nociceptive sensory afferents at the spinal level, and is involved in the expression of morphine analgesia in some circumstances (5, 17, 22). These data suggest that potentiation of 5HT function might be useful in the treatment of pain. 5HT function can be potentiated by tricyclic antidepressants and other monoamine uptake inhibitors, and both classes of drugs are used in the treatment of chronic pain, particularly when it is associated with depression (26). Because the synthesis of 5HT is controlled in part by the availability of its dietary precursor L-tryptophan (39), the use of tryptophan has been examined in several situations where potentiation of 5HT function may be therapeutic (41).

Experimental studies on the analgesic effect of tryptophan in human subjects and animals have yielded inconsistent results. In humans, tryptophan has been tested experimentally as an analgesic. Seltzer et al. (31) gave tryptophan or placebo to 30 normal subjects and looked at the response to electrical stimulation of dental pulp. The threshold for perception of pain was not altered by tryptophan, but pain tolerance was significantly increased. On the other hand, 14 days of tryptophan treatment (2 g per day) failed to alter radiant pain thresholds in groups of 20 female student volunteers (24). Tryptophan depletion, with a mixture of all the essential amino acids except for tryptophan (42), did not have any effect on the response of normal males in the cold pressor test, but completely reversed the analgesic effect of morphine (1).

Several studies have investigated the efficacy of tryptophan in clinical pain. King reported that tryptophan relieved the pain of patients in whom chronic pain had recurred after successful treatment by rhizotomy or cordotomy (21). Similarly, De Benedittis et al. (11) successfully treated patients suffering from deafferentation pain with 5-hydroxytryptophan, the metabolite of tryptophan and immediate precursor of 5HT. While these two studies were uncontrolled, patients continued the treatment for periods up to 13 months, and patients with longstanding pain tend to withdraw from treatment if it is ineffective. In a controlled trial, Seltzer et al. (29) found tryptophan to decrease clinical pain in patients with chronic maxillofacial pain. Tryptophan has also been reported to reduce pain 24 hours after endodontic surgery (36). No effect of tryptophan or 5-hydroxytryptophan was found in patients with disk disease (37) or with fibrositis syndrome (25). In a recent study, tryptophan given pre- and postoperatively did not effect pain development or analgesic consumption after third molar surgery (14). Finally, in a study on pain after abdominal surgery, higher tryptophan levels were associated with increased morphine requirements, and a trend to increased pain (16).

Animal studies, like the human studies, are inconsistent. In the formalin test in rats, an animal model of tissue injury pain, acute loading with tryptophan antagonized morphine (2). Tryptophan loading did not influence pain in the tail immersion test in rats (K. B. J. Franklin, personal communication). On the other hand, lowering brain tryptophan levels by administering an amino acid that competes with tryptophan uptake into brain antagonized morphine in the tail immersion test in rats (20).

From this literature, one condition in which all the studies with tryptophan or 5-hydroxytryptophan have shown a therapeutic effect is chronic pain associated with deafferentation or neural damage. We therefore tested the effect of tryptophan, given by itself, in an animal model of deafferentation pain, nerve lesion-induced autotomy. It has been suggested (3, 10, 38) that the self-mutilation, or autotomy, following denervation of a limb

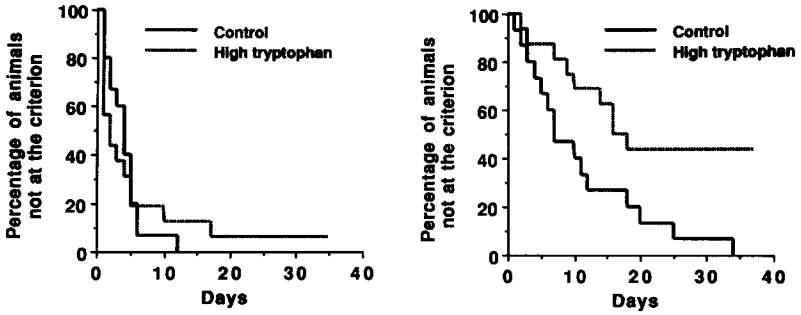

FIG. 1. Percentage of rats reaching the indicated criteria in the control and high-tryptophan groups. Each group consisted of 15 animals. Left panel: Survival plot showing the number of days it took the rats to begin chewing their nails. Right panel: Survival plot showing the number of days between the time the animals began chewing their nails, and the time they had amputated the three distal joints of at least one digit on the denervated paw.

in animals is a model of the dysesthesia experienced after amputation or nerve lesions in humans (19, 32, 35). Typically, autotomy follows a stereotyped, species-typical course. In rats, the animal begins to chew off its nails a few days after denervation. It then proceeds to chew off the digits, and may remove the whole foot if not sacrificed (3, 10, 38). Rats do not show signs of chronic pain such as irritability, weight changes or poor grooming, but have a tendency to interrupt ongoing behavior to vigorously attack the denervated paw (13). This behavior is consistent with the periodic paroxysms superimposed on a low level of dysesthesia that are described by individuals with amputation or nerve injury (35,40). Abnormal neural activity in regenerating axons is correlated with autotomy (7, 12, 28), and the behavior is exacerbated by sympathetic activity. It has also been suggested that the primary pathology is a loss of neurotrophic factors after denervation (8). Thus autotomy is a pathological condition caused by neural damage, and it is possible that enhancing 5HT function could attenuate it. Positive results would suggest the potential use of tryptophan in phantom limb pain in humans.

METHOD

All procedures were approved by the Ethics Subcommittee of the McGill University Animal Care Committee which operates under the guidelines of the Canadian Council on Animal Care.

Subjects and Tryptophan-Controlled Diet

Long-Evans rats (Charles River Canada, Inc., St. Constant, Québec) were obtained at 275–300 g. They were housed in group cages of 3 with food and water available ad lib. They were fed a tryptophan-free diet based on acid-hydrolyzed casein (Techlab), which was supplemented with either 0.15% or 0.75% tryptophan. The control level of tryptophan, 0.15%, was chosen because maximum growth in weanling animals requires at least 0.14% tryptophan in their diet, while adults need 0.11% (11). The experimental group was given a five-fold greater amount than the control group because a common dose of tryptophan given for therapeutic purposes in humans is 6 g per day, which is five times greater than the normal daily dietary intake of about 1.2 g per day (41). The diet is somewhat unpalatable and weight gain was slower than on laboratory chow. Rats were started on the diet 3 to 5 days before ligation and section of the sciatic and saphenous nerves, and kept on it until the termination of the experiment.

Nerve Ligation and Autotomy Evaluation

Rats were anesthetized with 30 mg/kg pentobarbital supplemented with ketamine/xylazine 3/0.3 mg/kg. The sciatic and saphenous nerves were exposed, ligated with 3-0 suture and cut distal to the ligation. The skin was sutured and the rats returned to the home cages. The completeness of the denervation was evaluated 1 day after surgery by gently pinching the animals' toes.

Autotomy was evaluated each day and any damage to the denervated paw was recorded. Animals were sacrificed when the 3 distal joints of one or more digits was chewed off. This criterion for terminating the experiment was chosen because it is common for a rat to chew off the anesthetic foot very quickly after it reaches this point, and bleeding may occur. The experiment was also terminated if a rat lost more than 10 g in a 24-hour period, suggesting illness or distress. This occurred in one instance in this experiment, and the data are entered into the survival analysis as censored on the day of sacrifice.

Biochemical Measurements

Rats were sacrificed when they reached the criteria for termination described above, or when 33–37 days had elapsed. Animals were anesthetized, between 10 a.m. and 12 a.m., with ketamine/xylazine (5/0.5 mg/kg), and the brain and a 10 mm section of the spinal cord at the level of the lumbar enlargement were removed. The tissue samples were stored at −70°C until they were assayed for tryptophan, 5HT and 5-hydroxyindoleacetic acid (5HIAA) by reverse phase high performance liquid chromatography with fluorometric detection (4).

Data Analysis

The autotomy data was subjected to survival analysis (BMDP1L). When appropriate, the Fisher Exact Probability test was used. Differences between biochemical data in the two groups were compared using Student's *t*-test. Relationships between various variables were determined using Spearman's product-moment correlations.

RESULTS

Overall, the high-tryptophan diet reduced the number of rats that reached the sacrifice criterion of removing the distal 3 joints

TABLE 1

BRAIN AND SPINAL CORD INDOLES IN SAMPLES TAKEN ON THE DAY CRITERION WAS REACHED, OR 33–37 DAYS AFTER DENERVATION

	N	Tryptophan (μg/g tissue)	5HT (ng/g tissue)	5HIAA (ng/g tissue)
Brain				
Control	15	4.43 ± 0.74	490 ± 61	281 ± 47
High tryptophan	15	5.77 ± 1.12‡	574 ± 45‡	340 ± 51†
Spinal Cord				
Control	14	4.44 ± 0.97	746 ± 92	310 ± 52
High tryptophan	15	5.42 ± 1.17*	841 ± 84†*	368 ± 57†

All values are given as mean ± SD. *$p<0.05$; †$p<0.01$; ‡$p<0.001$ against control values.

from one or more digits (Fisher Exact $p=0.047$). The high-tryptophan diet did not delay the tendency of rats to begin chewing their nails on the denervated paw, and all except one rat chewed their nails (Fig. 1, left panel; Generalized Savage statistic<0.01, $p>0.9$). However, the length of time between when animals began to chew their nails, and when they reached the criterion (amputating the 3 distal joints of at least 1 digit) was increased significantly by tryptophan supplementation (Fig. 1, right panel; Generalized Savage statistic = 5.42, $p<0.02$). Of the rats that reached the criterion, 7/14 control rats and 1/9 high-tryptophan rats amputated all 5 digits and, at the time criterion was reached, there was a trend for the total number of joints amputated to be higher in the control group (Fisher Exact test; $p=0.06$). Most of the rats that failed to autotomize (5/7) suffered some tissue damage to the skin of the denervated paw, probably due to contact with the wire metabolic cages that were necessary to feed the powdered diets. These wounds healed, except in one rat which was sacrificed when severe cellulitis developed (data entered as "censored" on day of sacrifice for survival analysis).

The brain and spinal cord tryptophan, 5HT and 5HIAA levels are shown in Table 1. The high-tryptophan diet produced 30% and 23% increases in the brain and spinal cord tryptophan levels respectively. 5HT and 5HIAA were increased to a slightly lesser extent, but all the increases in CNS indoles due to tryptophan supplementation were statistically significant. The rats were killed during the daytime. However, food intake, and therefore tryptophan intake in the supplemented group, is greatest during the night. Therefore, the biochemical data suggest that indoleamine metabolism was elevated for the full 24 hours per day.

Brain and spinal cord tryptophan were significantly related in rats on both diets (control r = .78; high tryptophan r = .88, $p<0.01$). There was no significant relationship between brain and spinal cord levels of 5HT or 5HIAA. In brain, levels of the three indoles tended to be correlated (r's ranged from .41 to .80, 5 out of 6 p's<0.05). In spinal cord samples, only the relation between tryptophan and 5HIAA in the rats fed high-tryptophan diets was strong (r = .71, $p<0.01$).

At no time did body weight differ significantly between the two groups, suggesting that the extra tryptophan had no effect on food intake or energy metabolism. Survival time was correlated with body weight for the two groups taken together (r = .87, $p<0.01$), as animals which survived longer had more time to grow. To rule out the possibility that body weight might have been related to the behavioral response, independent of its alteration over time, we looked at the relationships between indole levels and both body weight and time. Out of 12 correlations between body weight and indoles (control/high tryptophan, brain/spinal cord and 3 indoles), only one was significant at the .05 level. The pattern of correlations between survival time and indole levels was similar (1/12 correlations significant at $p<0.01$). These data indicate that the indole levels were stable during the period of the experiment, and that body weight was not related to response, independent of time.

DISCUSSION

The data presented here demonstrate that a moderate increase in the amount of tryptophan ingested reduces the incidence of autotomy following denervation of a limb. The severity of autotomy is also marginally reduced. Because virtually all rats chewed on their nails following denervation, one could speculate that the high-tryptophan diet does not abolish the dysesthesia produced by denervation. Instead, the intensity of attacks on the denervated paw was reduced to the extent that the nails regrew, and tissue damage due to contact with the wire cages healed in most tryptophan-treated animals. This suggests that the treatment reduced the intensity and duration of the dysesthesia that the animals experienced. An attractive explanation of this is that increased 5HT function in the spinal cord resulted in increased gating of nociceptive afferents.

Our results are consistent with reports that tryptophan supplementation ameliorates chronic pain following denervation or nerve injury in humans (11, 21, 30). The data emphasize that the role of 5HT is different in different pain tests in animals: in the formalin test, tryptophan loading attenuates the effects of morphine but has no effect on baseline pain (2); in the tail immersion test, tryptophan depletion attenuates the effects of morphine (20); finally in the test of chronic pain associated with denervation in the present study, tryptophan, by itself, reduced the animals' response.

Denervation following nerve injury or amputation of a body part usually results in sensation of a phantom that is described in distinct and specific terms (15,27). In a survey of 2,000 military amputees, Sherman and his colleagues found that phantom sensations were perceived as distressing by about half the subjects (35). Studies of civilian amputees also suggest that between 50 and 80% of amputees experience unpleasant phantom sensations (19,32). The commonly experienced sensations are probably distinct from causalgic pain associated with abnormal sympathetic activity, which suggests reflex sympathetic dystrophy (18, 23, 35). According to Sherman and his colleagues, it is common for patients not to mention disagreeable phantom sensations in conversations with their physicians because of the fear that the pain will be labeled as psychological (35). Those that do complain have received many treatments, none of which are particularly efficacious (33,34). In fact, the more often a treatment is used, the more likely it is to be reported to be ineffective (34). The present study suggests that the efficacy of tryptophan in phantom limb should be tested. Although a serious side effect, the eosinophilia-myalgia syndrome, has recently been reported, this seems to be due to a contaminant of tryptophan that was found in a few batches from a particular manufacturer (6). In general, tryptophan seems to be remarkably safe when used as a drug, and it is associated with few side effects (41). Thus tryptophan should be tested in phantom limb pain for both theoretical and practical reasons.

ACKNOWLEDGEMENT

This work was supported by the Medical Research Council of Canada.

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