SEROTONIN INVOLVEMENT IN A MOTOR DISORDER OF SCOTTISH TERRIER DOGS

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Summary

The effect of altering the concentration of 5-HT or the catecholamines upon an inherited neurological condition of Scottish terrier dogs which is characterized by episodes of muscular hypertonicity was assessed in a blind study. Alpha-methyl-para-tyrosine and imipramine did not modify the condition. Amphetamine sulfate induced episodes; however, the episode was generally of shorter duration than the behavioral effect. The severity of the clinical rating was markedly increased by p-CPA. This increased severity was reduced by 5-HTP administration. The peripheral serotonin antagonist ylamlidine tosylate did not alter the severity of the disease. Nialamide and 5-HTP had a significant beneficial effect. The increase in severity of the disease which follows a decrease in 5-HT coupled with a decrease in severity with an increase in 5-HT suggest certain serotonergic neurons are involved in modulation of skeletal muscle tone.

Introduction

An inherited central nervous system disorder characterized by episodes of progressive muscular hypertonicity leading to postural and locomotive abnormalities appears to be unique to the Scottish terrier breed of dogs (1,2). The dogs appear normal at rest, however, if excited, clinical signs are observed within a few minutes. Once initiated, the clinical signs progress in severity, reach a peak, then remain constant. If the episode inducing factor is removed, there is a progressive remission of clinical signs. From the characteristics of the disease, coupled with the absence of histopathologic and clinical chemistry abnormalities, we hypothesized that this disease results from either an accumulation or a functional deficiency of a compound within the central nervous system, (3). The experiments reported herein were
designed to study the influence of various pharmacological agents, which affect the putative neurotransmitters dopamine (DA), norepinephrine (NE), and serotonin (5-HT).

**Methods**

Ten affected and five normal adult Scottish terrier dogs were used. The following drugs were employed: amphetamine sulfate (Amfetsul™) which is thought to release the catecholamines from nerve terminals (4); imipramine, which has been reported to interfere with the neuronal reuptake of catecholamines (5); Alpha-methyl-para-tyrosine (AMPT) methylester, which decreases brain NE and DA levels possibly by inhibiting tyrosine hydroxylase (6,7); nialamide, a monoamine oxidase inhibitor which, at a dose of 50-75 mg/kg in the dog has been shown to produce nearly a fourfold increase in brain stem 5-HT with only a slight increase in NE (8); p-chlorophenylalanine (p-CPA) which decreases brain 5-HT concentration presumably by inhibiting tryptophan hydroxylase (9,10,11); 5-hydroxytryptophan (5-HTP) which passes the blood-brain barrier and is then converted to 5-HT by the enzyme 5-HTP decarboxylase (12); p-CPA followed by 5-HTP to reverse the p-CPA induced reduction of 5-HT (13); and xylamidine tosylate which is a powerful peripheral but weak central 5-HT antagonist (14). All drugs except amphetamine and imipramine were evaluated in a blind study with the observer being unaware of the specific treatment.

Ten affected and one normal dog were randomly designated to receive either a placebo or one of the above drugs. Treatment was given over a five day period with the drug or placebo administered four hours prior to evaluating the disease unless otherwise stated. The dogs were withdrawn for two days and the process repeated until at least five affected dogs and one normal dog were tested with each drug. Dogs which received nialamide, AMPT or p-CPA were given a placebo for the next five day test. Prior to commencing drug administration, the dogs were evaluated for five days following a placebo. Those drugs which affect the disease were later evaluated on the remaining normal dogs. To evaluate the disease, episodes were elicited by having each dog
exercise freely near a caged ferret. This was found to be the most consist-
ent and reliable method of eliciting an episode. The ferrets had been
raised near the dogs so they were not alarmed at the sight of the Scottish
terrier dogs and the cage was constructed so that it was impossible for
the dog and ferret to come into contact with one another. The time of
exercise required to evoke an episode and the severity of the episode was
assessed and given a numerical rating. The sum of the numerical rating of
time and severity is reported as the clinical rating for that episode.
However, if a drug affected one parameter without affecting the other they
were reported separately. The test was usually recorded on video tape or
movie film. The time required to elicit an episode was divided into the
following groups: 0 - No clinical signs in 15 minutes; 1 - clinical signs
first observed between 10 and 15 minutes; 2 - clinical signs first observed
between 1 and 10 minutes; 3 - clinical signs first observed within one minute;
and 4 - clinical signs present when first observed. The severity of the
episode was rated as follows: 0 - no clinical signs observed during the
test; 1 - abduction of front limbs or mild arching of the back and stiff-
legged gait; 2 - marked back arching and stiff-legged gait; 3 - hind limbs
rigid with forward progression due entirely to front limb movement; and 4 -
piller like stance with complete immobility. The test was terminated either
when clinical signs were constant or after fifteen minutes.

Prior to and during the assessment of the clinical rating each dog
was evaluated for behavior modification. The observer specifically noted in
non-disturbed animals, if there was an increased locomotive activity, anxiety,
altered aggressiveness, altered attention span or depression. In addition,
any overt behavior change was reported.

Four affected dogs were anesthetized with thiamyl sodium and a 2 ml
cerebrospinal fluid (CSF) sample was withdrawn from the cisterna magna and
immediately frozen for subsequent analysis of either 5-hydroxyindoleacetic
acid (5-HIAA) or Homovanillic acid (HVA). Following administration of
AMPT methyl ester (150 mg/kg i.p.), nialamide (60 mg/kg/day for 5 days) and p-CPA (100 mg/kg/day for 3 days), 5-HIAA or HVA concentration in cisternal CSF was determined. 5-HIAA was measured according to the method of Ashcroft and Sharman (15) and HVA by the method of Gerbodie and Bowers (16).

Results
Amphetamine sulfate, 0.5 - 2.0 mg/kg, injected intramuscularly (i.m.) or intravenously (i.v.) into seven affected Scottish terrier dogs induced, within fifteen minutes, an episode that was indistinguishable from that induced by excitement. The dose required to elicit an episode and the duration of the episode in a given dog was variable, as shown in an affected dog which received amphetamine sulfate (0.5 mg/kg i.m.) on four different occasions. Following each injection the dog was isolated and observed every hour for four hours. In one instance the clinical signs were not observed during the four hours. The other three trials resulted in clinical signs persisting for 1½ hours, 3½ hours and for 4 hours. Five normal Scottish terriers injected with amphetamine sulfate did not show signs of the disease. Following amphetamine injection, pupil dilation along with anxiety and increased locomotion were observed throughout the four hour observation period.

Imipramine was administered to two affected and one normal dog at an initial dose of 4 mg/kg. The dose was increased 2 mg/kg/day until a final dose of 12 mg/kg was achieved. Imipramine had no observable effect on any dog.

Administration of the methyl ester of AMPT (150 mg/kg i.p.) to six affected dogs as a single dose, four, eight, sixteen or twenty hours prior to the evaluation neither modified the time required to induce an episode nor the severity of the episode. Larger doses of AMPT were not given because preliminary studies in mongrel dogs receiving 200-250 mg/kg i.p. resulted in interstitial nephritis and hemorrhagic ulcerative gastritis.
CSF HVA was reduced from 132 ng/ml to 72 ng/ml four hours after AMPT administration. The cisternal CSF concentration of HVA in 4 nonaffected nontreated dogs was 128 ng/ml.

Figure 1 represents the daily assessment of the clinical rating of the 10 affected dogs in this study during placebo administration.

Oral nialamide (30 mg/kg/day b.i.d.) to six affected dogs for five days significantly altered the clinical rating (Figure 2). Improvement in the disease was evidenced both in a decrease in the severity of clinical signs and an increase in time required to induce the episode. Nialamide did not appear to alter the behavior of either the nonaffected or affected dogs. After five days of nialamide, cisternal 5-HIAA was decreased from 55 ng/ml to 20 ng/ml and cisternal HVA from 132 ng/ml to 66 ng/ml.
Clinical Rating of Affected Dogs Receiving Nialamide (60 mg/kg/day orally). Values Represent Mean ± S.E.M.

FIG. 2

Treatment of five affected dogs with p-CPA (100 mg/kg/day orally) for five days, markedly increased the rating of the episode(s) (Figure 3). The increase in severity reached a peak on the third day, then remained constant. The effect of the drug was so pronounced that one dog was immobile when first observed on the third day, and was removed from the experiment.

p-CPA has been administered to five additional affected dogs, which were not in the blind study, with similar results. With the exception of one dog, who exhibited a slight hypersexuality, no behavioral changes were observed. Five nonaffected Scottish terriers received p-CPA for five days without observable effects. After three days of p-CPA, 5-HIAA concentration in
cisternal CSF was reduced from 55 ng/ml to 16 ng/ml.

![Clinical Rating](image)

**FIG. 3**

Clinical Rating of Affected Dogs Receiving p-CPA (100 mg/kg/day Orally). Values Represent Mean ± S.E.M.

Administration of 5-HTP, i.p., at an initial dose of 5 mg/kg followed by an increase of 1 mg/kg/day for 4 days did not alter the disease in five dogs tested.

The experiment was repeated with four of the most severely affected dogs in the colony receiving 5-HTP (15 mg/kg i.p.) two hours prior to evaluation. 5-HTP increased the time required to elicit an episode in dog #1 without affecting the ultimate severity (Table 1). However, of the remaining 3 dogs, two were unaffected and the third became timid and with-
drawn such that a valid assessment could not be accomplished. The experiment was repeated, altering the dose by 2.5 mg/kg, until a consistent effect on the disease was observed. As shown in Table 1, the time required to elicit an episode was increased but the ultimate severity remained unchanged.

Table 1
Assessment of Affected Dogs Receiving 5-HTP

<table>
<thead>
<tr>
<th>Dog Number</th>
<th>Dose (mg/kg)</th>
<th>Clinical Rating Prior to 5-HTP</th>
<th>Clinical Rating Following 5-HTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>15</td>
<td>0.25 (8) ³ 3</td>
<td>5.0 (4) 3</td>
</tr>
<tr>
<td>2.</td>
<td>25</td>
<td>0.80 (5) 3</td>
<td>3.0 (2) 3</td>
</tr>
<tr>
<td>3.</td>
<td>20</td>
<td>2.50 (5) 3</td>
<td>5.0 (2) 3</td>
</tr>
<tr>
<td>4.</td>
<td>12</td>
<td>1.20 (5) 3</td>
<td>3.5 (2) 3</td>
</tr>
</tbody>
</table>

1. Time required to elicit clinical signs.
2. Severity based on a 0–4 scale.
3. Data presented as the mean. Number of trials in parentheses.

5-HTP was effective in reducing the p-CPA increase in clinical rating. Six affected dogs were given p-CPA (100 mg/kg orally) for three days and on the third day 15 mg/kg of 5-HTP was administered (i.p.). 5-HTP increased the time required to induce the episode threefold (Figure 4). However, 5-HTP did not decrease the ultimate severity of the episode.

Xylamidine tosylate (0.15 mg/kg i.m.) was administered to four affected dogs and the dogs tested 0.5, 1, and 2.5 hours following administration. This treatment did not alter either the time required to induce an episode or the severity of the episode.
The Effect of 5-HTP (15 mg/kg) on the Time in Minutes Required to Elicit an Episode and the Severity of the Episode, As Based on a Scale of 0-4, in Affected Dogs Receiving p-CPA (100 mg/kg/day.)

**Discussion**

Drugs which altered catecholamines did not significantly modify this disease. Although amphetamine sulfate did elicit an episode, the remission of clinical signs while it was still pharmacologically active suggest amphetamine sulfate may be acting at a site other than where the abnormality occurs. The inability of AMPT to modify this condition may be interpreted that brain catecholamines were not altered by AMPT, that catecholamines were not significantly decreased by AMPT or that catecholamine function is normal in affected dogs. The first alternative is unlikely since numerous laboratories have replicated the original finding of Spector, Sjoerdsma and Undefriend (6) regarding the preferential catecholamine depleting action.
of AMPT. In this study cisternal CSF HVA was decreased approximately 50%. CSF HVA measurements can provide information relative to metabolism of the parent amine in the CNS as indicated by numerous studies which have demonstrated reduced values for CSF HVA (17,18,19) in parkinsonism where a decreased DA is present. The apparently normal concentration of cisternal HVA in affected dogs suggests that the DA is normal in the affected dogs.

In regard to the second alternative, if the motor activity exhibited in the disease resulted from a catecholamine abnormality, significantly reducing their concentration should modify the clinical signs. Therefore, it would appear that the third possibility is the more likely.

Drugs which altered 5-HT concentration within the central nervous system consistently altered the severity of the disease. Decreasing 5-HT concentration with p-CPA increased the severity of the condition as indicated by the increase in severity of clinical signs and the decrease in time required to elicit an episode. p-CPA appears to modify this disease by decreasing 5-HT, as its effect can be reversed by 5-HTP administration. Since p-CPA alters 5-HT metabolism in the periphery as well as the brain, these extra CNS factors cannot be discounted. However, blocking the peripheral 5-HT receptors with xylamidine tosylate did not alter the disease.

Additional evidence for a 5-HT involvement is provided by experiments where 5-HT concentration was increased by either nialamide or 5-HTP. 5-HTP increased the time required to elicit an episode without altering the ultimate severity of the episode. Nialamide progressively lowered the clinical rating of the disease, i.e., nialamide decreased the severity of the episode and increased the time required to elicit an episode. Since monoamine oxidase is the major enzyme in 5-HT degradation, a decrease in 5-HIAA with a corresponding increase in 5-HT, should occur following nialamide administration. The inability of 5-HTP to modify the ultimate severity of the episode may result from a greater increase in neuronal
5-HT concentration following nialamide than following 5-HTP. Administration of 5-HTP will increase non-neuronal 5-HT, presumably due to the ubiquitous disposition of aromatic amino acid decarboxylase in the CNS (20, 21). Bogdanski et al. (12) reported that a 60 mg/kg dose of 5-HTP produced an approximate sixfold increase in brain stem 5-HT. However, this dose of 5-HTP was considerably larger than that used in this study. On the other hand, Maling et al. (8) reported a fourfold increase in brain stem 5-HT with a similar dose of nialamide as was used in this study. In addition, they reported that NE was only slightly elevated. Although the concentration of NE may not have been significantly modified following nialamide administration, a reduction in cisternal HVA as well as 5-HIAA indicated brain DA concentration was altered. Therefore, the decrease in severity following nialamide cannot be entirely attributed to an increase in 5-HT.

Drugs which increased CNS 5-HT concentration decreased the clinical rating while those reducing CNS 5-HT concentration increased the clinical rating. These data suggest two alternate hypotheses. First, a functional or absolute deficiency of 5-HT exists in the affected dogs with the clinical signs resulting from the inability of 5-HT to exert a postsynaptic effect. A short period of rest allows for a physiological increase in the transmitter to a level adequate to carry on normal function.

In 1968 it was suggested that 5-HT has a trophotrophic or sedative action on the central nervous system (22). Since then, an accumulation of evidence suggests that both external and internal thresholds are mediated by 5-HT mechanisms (23, 24, 25). As a second alternative, 5-HT is normal and 5-HT neurons are one factor responsible for suppressing clinical signs resulting from a defect which is not directly centered in the serotonergic pathways. Although there appears to be a relationship between the severity of the disease and serotonin concentration it is not known if a serotonin abnormality is the primary defect per se or if altering serotonin influences neuronal events in
Histochemical evidence has revealed that 5-HT containing cell bodies in the caudal raphe nuclei give rise to axons which descend to the spinal cord (26). Studies on the significance of the descending serotonergic system in motor regulation suggest that these neurons markedly increase motorneuron excitability (27,28,29) and inhibit flexor reflexes (30,31). A serotonergic increase in motorneuron excitability is not compatible with the observed increase in muscle tone in affected dogs when 5-HT concentration is decreased. If descending 5-HT neurons inhibit flexor reflex afferents then reducing this inhibition by decreasing 5-HT should excite flexor and inhibit extensor motorneurons. Electromyograms recorded during episodes (3) as well as the clinical signs do not indicate that such a situation exists in this condition.

Clineschmidt and Anderson (32) reported an inhibition of the monosynaptic reflex following electrical stimulation of the raphe nucleus in decerebrate cats. This inhibition could be blocked by 5-HT antagonists. The conduction velocity of the neurons causing the inhibition and the route of administration of the antagonist suggests that the inhibition was mediated by a 5-HT releasing interneuron in the spinal cord. Although evidence has not been presented in this paper to support the presence of an inhibitory 5-HT containing interneuron in the spinal cord, an inhibitory action of 5-HT is consistent with our findings.

Regardless of whether or not this disease is a 5-HT mediated disorder, the increase in severity when 5-HT concentration is reduced and the decrease in severity when 5-HT concentration is elevated suggest certain 5-HT neurons have an inhibitory effect on motor neuron activity.

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References

10. S.S. TENEN, Psychopharmacol. 10 204 (1967).