well (DeLahunta 1983). Since the nigro-striatum effect appears to be mediated by the medullary extensor muscle inhibition centre (Bourke 1987), it seems most likely that there has been an additional tryptaminergic dysfunction in the medial medullary reticular formation. The effect of this would be transmitted by either descending serotonergic or descending cholinergic motor neurones that innervate gamma motor neurones throughout the spinal cord.

To act successfully as neurotoxins in *T terrestris* staggers, which is a chronically progressive and irreversible syndrome, harmane and norharmane would need to be accumulated in the affected sheep. We propose that this accumulation occurs in the affected upper motor neurones and that these neurones are tryptamine-associated. We further propose that the chemical structure of harmane and norharmane is such that they are preferentially taken up by, and stored within, the tryptamine-associated neurones of the central nervous system. This concept has already been demonstrated for the structurally related compound 1-methyl-4-phenyl pyridine in the neurones of the dopaminergic nigro-striatal projection (Snyder and D'Amato 1985). We suggest that they are gradually released from their cytoplasmic storage site, enter the nucleus, and interact with a specific neuronal gene DNA sequence. DNA interaction has been demonstrated *in vivo* for both harmane and norharmane (Hayashi *et al* 1977; Lau and Luh 1979). The gene site would be one specific for the production of an enzyme essential to the synthesis of tryptamine, notably aromatic L-amino acid decarboxylase. Gene switches can operate through feedback mechanisms to control the intracellular synthesis of end products (Alberts *et al* 1989; De The *et al* 1990). The structural similarity between harmane, norharmane and tryptamine would enable the toxins to mimic tryptamine and either switch on or switch off the gene site. The structural difference would prevent the toxins from being dislodged, and as a consequence the gene site would no longer be able to switch back.

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Mechanisms underlying *Phalaris aquatica* "sudden death" syndrome in sheep

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SUMMARY: Twenty outbreaks of *Phalaris aquatica* "sudden death" syndrome in sheep were investigated between 1981 and 1991. Four were confirmed and one was suspected, to be a cardiac disorder; 5 were confirmed and 3 were suspected, to be a polioencephalomalacic disorder; the aetiology of the remaining 7 outbreaks could not be determined. Potentially toxic levels of hydrocyanic acid (20 to 36 mg/100 g) were measured in the 3 toxic phalaris pastures tested. The measurement of potentially toxic levels of nitrate nitrogen (2920 μg/g) in toxic phalaris pastures by others, was noted. It is suggested that phalaris "sudden death" syndrome could have as many as 4 different underlying mechanisms, and that these might reflect the presence in the plant of a cardio-respiratory toxin, a thiaminase and amine co-substate, cyanogenic compounds, and nitrate compounds.

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Introduction

Intoxication of sheep grazing *Phalaris aquatica* pastures was first documented in 1942 by McDonald. Initial accounts described a nervous syndrome called "staggers". By 1961, an apparently unrelated syndrome called "sudden death" was also recognised (Moore *et al* 1961). This was subsequently shown to be due to a cardiac dysfunction (Gallagher *et al* 1964). The clinical signs displayed by affected sheep in these outbreaks of "sudden death" syndrome have been described in only 2 first-hand accounts (Moore *et al* 1961; Gallagher *et al* 1964). These referred to a sometimes fatal, cardio-respiratory disorder, of sudden onset and without any associated nervous signs, which was precipitated when sheep grazing phalaris pastures were mustered or otherwise disturbed. The first inconsistency in the reporting of this syndrome appeared when Moore and Hutchings (1967) described sheep being found dead in the morning, within 16 h of a flock being depastured on phalaris, and without associated forced exercise or other disturbance. This inconsistency was overlooked, and the popular assumption developed that all outbreaks of phalaris "sudden death" syndrome involve the cardiac dysfunction described by Moore *et al* (1961) and Gallagher *et al* (1964). In view of such inconsistencies in some

outbreaks investigated by our laboratory, a review was undertaken of all cases of this syndrome on our files over a 10-year period.

This report documents outbreaks of phalaris "sudden death" syndrome investigated at the Regional Veterinary Laboratory, Orange, during the period 1981 to 1991. It establishes the existence of a polioencephalomalacic presentation, as well as a cardiac presentation, of this syndrome, and raises the possibility that phytogenous cyanide and nitrate poisonings might be responsible for other presentations.

Materials and Methods

Between 1981 and 1991 twenty outbreaks of phalaris sudden death syndrome in sheep were investigated. These were categorised into different presentations of the disease on the basis of clinical signs and microscopic changes in brains from affected sheep. A cardiac presentation and a polioencephalomalacic presentation were established. The cardiac presentation was defined as the sudden onset of a cardio-respiratory disorder, without any associated nervous signs, precipitated in sheep grazing *phalaris pastures* when mustered or otherwise disturbed. Affected sheep collapsed, some recovered soon after, others died after a short period of acute respiratory distress; the mucous membranes and skin became cyanotic during brief episodes of ventricular fibrillation and cardiac arrest. An additional diagnostic requirement was the absence of any microscopic changes in the brains of affected sheep. The polioencephalomalacic presentation was defined as the presence of dead sheep on phalaris pastures, together with the following signs in affected survivors: blindness, depression, aimless wandering, head pressing, head and body tremors, opisthotonus, recumbency, twitching of the face and ears, teeth grinding, salivation, intermittent limb paddling, and either cerebral convulsions or coma. An additional diagnostic requirement was the presence of microscopic changes consistent with polioencephalomalacia in the brains of affected sheep.

Brains from affected sheep were fixed in 10% buffered formalin and sectioned transversely at the following levels: forebrain, optic chiasma, mamillary body, superior colliculus, pons, cerebellum and medulla. These sections were cut at 6 μm and stained with haematoxylin and eosin. The concentration of hydrocyanic acid (HCN) in toxic phalaris plant material was measured in 3 outbreaks, by the alkaline titration method as described in AOAC methods of analysis (Anon 1965).

Results

Four of the 20 outbreaks investigated were categorised as the cardiac presentation of phalaris "sudden death" syndrome; another was suspected, on the basis of clinical signs, but brain tissue was not collected. Five outbreaks were categorised as the polioencephalomalacic presentation; 3 others were suspected, on the basis of clinical signs, but brain tissue was not collected. In the other 7 outbreaks sudden deaths occurred, but no affected live animals were found, and brains were collected on only 2 occasions. No significant microscopic changes were demonstrated in these brains.

Cardiac Presentation

These outbreaks occurred between late January and late April in several years. Sheep aged from 6 months to 6 years were affected. The flock prevalence was consistently less than 1%; most affected sheep died. The precipitating factor in each case was mustering the flock. The clinical signs were cardio-respiratory, as defined above (Materials and Methods). The affected flocks had been grazing the toxic phalaris pastures for at least 2 weeks before the outbreaks occurred. The cyanide content of toxic pastures, measured in only one outbreak, was 36 mg/100 g of dry plant.

Polioencephalomalacic Presentation

Outbreaks occurred between late May and late June in several years and affected 30 to 250 sheep aged from 9 months to 6 years. The flock prevalence ranged from 1% to 14% (mean 6%); very few affected sheep recovered. Each outbreak occurred within 12 to 48 h of hungry sheep being placed on previously spelled phalaris pastures. The first manifestation was sheep being found dead in the paddock. In 3 outbreaks the sheep had just been moved off paddocks that had been eaten bare. One of these flocks was being managed by a rotational grazing system. In one outbreak the affected flock had been purchased in a saleyard and then transported 150 km immediately beforehand. In another outbreak the affected flock had been held in bare yards for 24 h beforehand. In all cases the toxic phalaris pastures were short, sparse, dry weather affected, and freshly shooting. In one outbreak sheep were observed to pull up whole plants and eat the roots as well as the aerial parts.

The clinical signs observed (see Materials and Methods) in the few affected survivors in each case were typical of those seen in cases of ovine thiamine-associated polioencephalomalacia (Blood and Radostits 1989), and there were consistent histological abnormalities in the brains of affected sheep. In the cerebral cortex there was a very acute reaction, with extensive perivascular and perineuronal vacuolation of the deep laminae and in some cases patchy involvement of the superficial laminae. Many neurones were shrunken and acidophilic. However, because of the rapid development of the lesions, there was no vascular endothelial proliferation or gitter cell response to the neuronal necrosis. In the cerebellum there was in some cases extensive cytoplasmic vacuolation of small neurones within the Purkinje cell layer, and some vacuolar degeneration of the Purkinje cells themselves. Brains from affected survivors had more advanced degenerative changes than those from dead sheep.

Other Outbreaks

In late September 1982, 8 of 800 adult sheep were found dead while grazing a phalaris pasture. In mid-June 1984, 4 of 50 adult sheep were found dead at pasture on phalaris. In mid-May 1986, 4 of 100 adult sheep were found dead within 48 h of being placed on a short, freshly shooting, previously spelled phalaris pasture. In late January 1989, 11 of 860, six-month-old sheep were found dead within 16 h of being placed on a short, freshly shooting, dry weather affected phalaris pasture. The sheep had been transported 600 km and were then unloaded onto the toxic pasture, which had a HCN content of 20 mg/100 g of dry plant. In mid-April 1989, 4 of 500 adult sheep were found dead within 48 h of being placed on a short, freshly shooting, previously spelled phalaris pasture containing 23 mg HCN/100 g of dry plant. In mid-June 1991, 150 of 1000 adult sheep were found dead within 16 h of being placed on a short, dry weather affected, freshly shooting, phalaris pasture. This flock was being managed by a rotational grazing system. In all of these outbreaks there was sudden death without premonitory signs, and routine field and laboratory examinations, which included necropsy, bacteriology, histopathology, and biochemical screening for metabolic diseases, failed to support an alternative diagnosis to phalaris "sudden death" syndrome, and therefore phalaris-associated sudden death was surmised.

Discussion

The results obtained in this investigation indicate that more than one disease mechanism underlies phalaris "sudden death" syndrome. Outbreaks categorised as the cardiac presentation are identical with those described by Moore *et al* (1961) and Gallagher *et al* (1964). Outbreaks categorised as a polioencephalomalacic presentation were supported as such on both clinical and neuropathological grounds. This presentation has not previously been recognised as such, although the account of

Moore and Hutchings (1967) may well have combined cardiac and polioencephalomalacic presentations, without recognising the difference, and the inconsistency with Moore *et al* (1961). The nervous signs displayed by the few surviving affected animals in these polioencephalomalacic outbreaks were different from those described for cases of the nervous syndrome, "phalaris staggers" (Bourke *et al* 1988, 1990).

In 7 of the 20 outbreaks of phalaris sudden death investigated, neither the cardiac presentation nor the polioencephalomalacic presentation could be confirmed. This was because there were no survivors in which clinical signs could be observed, and additionally in 5 outbreaks brains were not collected for microscopic examination. It is possible that some of these outbreaks may have involved other disease mechanisms. One possibility could be cyanide toxicity, since potentially toxic concentrations of HCN (20 mg/100 g or greater) were measured in the 2 pastures sampled. However, in outbreaks of the cardiac presentation an even higher concentration of HCN was recorded in the one pasture sampled. This could suggest that cyanide is not involved in phalaris "sudden death" syndrome, or that it can contribute to the cardiac presentation. Potentially toxic concentrations of HCN at the commencement of the phalaris growing season have been recorded in Argentina (Gaggino *et al* 1965). Another possible cause of phalaris "sudden death" could be nitrate poisoning. Phalaris pastures can attain nitrate nitrogen concentrations of 2920 μg/g (Moore and Hutchings 1967), and the potentially toxic concentration for sheep is 1000 μg/g or greater (Moore and Hutchings 1967). In both cyanide and nitrate intoxications, onset is sudden and most affected animals die. However, survivors would show similar signs, namely, disturbed cardio-respiratory function, muscle fasciculations, weakness, collapse, and coma or cerebral convulsions (Seawright 1989). With cyanide and nitrate toxicities the flock prevalence is usually high, and deaths usually occur in the first 48 h after introduction to the toxic pasture. Significant brain pathology has not been demonstrated in affected animals.

In this investigation the cardiac presentation of phalaris "sudden death" occurred less commonly and less disastrously than other presentations of the disorder. It appeared to occur sporadically in flocks that had adapted to grazing a particular phalaris pasture. Disturbing the flock, and consequently forcing exercise, seemed to be an important precipitating factor. Outbreaks of this presentation commonly occurred in the initial few months of the plant's growing season. The causal agent remains an enigma as none of the groups of compounds so far identified in phalaris has been shown to cause the described specific cardio-respiratory effect (Bourke *et al* 1988, 1990). The toxin responsible must act on either the cardio-respiratory centres of the medulla oblongata, or the terminations of the vagus nerve in the heart. Gallagher *et al* (1964) determined that the effect was one of arrhythmic tachycardia followed by ventricular fibrillation. Phenylethylamines have been identified in phalaris extracts (Frahn and O'Keefe 1971), and some related chemical structures, such as ephedrines and amphetamines, can exert cardio-respiratory effects. Pseudoephedrine, for example, when administered parenterally to sheep at 30 mg/kg, caused a transient, delayed onset, cardio-respiratory dysfunction (CA Bourke unpublished). The related structure, 6-hydroxy amphetamine, would predictably display greater cardio-respiratory toxicity.

The polioencephalomalacic presentation of phalaris sudden death was characterised by high mortality during the first 48 h of sheep being depastured on toxic pastures. This is atypical for classical polioencephalomalacia in sheep. The affected flocks were invariably hungry and therefore presumably ate the phalaris rapidly. These outbreaks were largely restricted to a rather brief period between late autumn and early winter. The demonstrated neuropathology reflected the unusually rapid onset of the condition, and brains from affected survivors, rather than dead sheep, were preferred specimens, because they represented cases of slightly longer duration. If this presentation is thiamine-related, then to cause such a sudden onset of polioencephalomalacia among so many sheep phalaris would need to contain, or to be able to generate, a potent thiamine antagonist in these sheep. Ovine polioencephalomalacia is generally regarded to result from the combined ingestion of thiaminases and amine co-substrates, and thiaminase activity in plants will peak in certain months of the growth season (Cheeke and Shull 1985). Intoxication appears to depend more on the availability of co-substrates structurally capable of producing analogues that competitively inhibit thiamine in the central nervous system than on intra-ruminal destruction of thiamine (Edwin and Jackman 1982). Some of the alkaloids in phalaris may be able to supply amine structures ideal for this co-substrate activity.

Prevention of the cardiac form of phalaris sudden death will require the identification of the specific cardio-respiratory toxin in the plant, and the selection of cultivars free of it. The impact of the polioencephalomalacic form can probably be reduced if stock owners are advised not to put hungry sheep on phalaris pastures. This applies particularly to flocks managed by a rotational grazing system, to sheep held in yards or wool sheds for a day or longer, and to sheep that have been transported over long distances. If either cyanide or nitrate poisoning are contributory factors to phalaris "sudden death" syndrome, then the advice for avoiding polioencephalomalacia would be equally applicable. Veterinarians investigating outbreaks of phalaris "sudden death" syndrome are advised to record accurately the clinical signs displayed by any affected survivors, to collect brains from affected sheep for formalin fixation and microscopic examination, and to collect rumen contents, whole blood and aqueous humour from affected sheep, and toxic pastures, for cyanide and nitrate determinations. In this way a greater appreciation of the different presentations that this syndrome can take will be achieved.

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