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MYOTONIA AS A SIDE EFFECT OF DIURETIC ACTION

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1 Commonly used loop diuretics produce side effects in man which are similar to chemically induced myotonia. These diuretics have structural affinity with known myotonic agents.

2 We have observed EMG myotonia *in vivo* in leg muscles of rats treated with intravenous frusemide.

3 In the presence of several different diuretics, rat isolated diaphragm, soleus and extensor digitorum longus muscles as well as frog sartorius muscles produce typically myotonic contractions with relaxation times up to several seconds.

4 Intracellular recording of action potentials from diuretic-treated muscles reveals long lasting after-discharges following a brief electrical stimulus, again typical of chemically induced myotonia.

5 Having demonstrated a myotonic action of several diuretics we suggest a need for caution in using these drugs in persons with hereditary myotonia and a need to be aware of possible provocation of myotonia in subclinical cases. Myopathies and neuropathies which are known to result from chronic exposure to myotonic agents also need to be considered.

6 In our study, the diuretic, acetazolamide, unmasked subthreshold myotonia. This seems to be at variance with reports of its usefulness in the treatment of myotonia.

7 Diuretics should probably not be employed in the treatment of herbicide intoxication where their myotonic activity would be expected to add to the known myotonic activity of the herbicide.

Introduction

Well known side effects of diuretic treatment include muscle pain, cramps, tetanic spasms and weakness (Guay, Mercier & Plamondon, 1965; Wade, 1977; Lane & Mastaglia, 1978). It is usual to ascribe these symptoms to electrolyte imbalance, in particular, hypokalaemia and hypocalcaemia as a result of the disproportionate urinary potassium and calcium losses produced by many diuretics (Dargie & Dollery, 1975; Wade, 1977). However, similarity of symptoms has led us to question whether some of these side effects may not be a form of drug-induced myotonia caused by a direct action of the diuretics on skeletal muscle.

Structurally, the most potent of the diuretics are monocarboxylic aromatic acids: either substituted benzoic acids or phenoxyacetic acids. Compounds from both of these classes have previously been shown to induce myotonia in animals (Bucher, 1946; Moffett & Tang, 1968) and numerous cases of poisoning in man, with subsequent myotonic symptoms, have been reported (Berwick, 1970; Brandt, 1971; Prescott, Park & Darrien, 1979). Such agents have been shown to produce myopathic changes on chronic administration to animals (Rüdiger, Meerbach, Osske & Fischer, 1972; Danon, Karpati & Carpenter, 1978) as well as myoglobinuria and raised plasma levels of skeletal muscle enzymes implicating muscle damage after poisoning in man (Berwick, 1970; Prescott *et al.*, 1979).

Our findings indicate that a number of diuretics can directly induce myotonia in animals while others can potentiate subthreshold myotonia. There are consequent implications for the indiscriminate use of diuretic therapy.

Methods

In vivo studies were performed on female Wistar hooded rats of approx. 200 g weight. They were anaesthetized with pentobarbitone sodium (60 mg/kg i.p.) and a tail vein was exposed for subsequent frusemide injections. Electromyograms (EMGs) were recorded from gastrocnemius and tibialis anterior

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muscles with a concentric needle electrode (Medelec EE/NO1).

Isolated diaphragm, extensor digitorum longus and soleus muscles from similar rats were used *in vitro*, bathed in synthetic interstitial fluid (SIF, Bretag, 1969) at 37°C. Isometric tension was recorded with a Grass FT03 strain gauge and a Beckman Dynograph curvilinear penwriter. Muscles were stimulated directly once every 10 min with a just supramaximal train of three pulses, each of 1 ms duration, at a frequency of 225 Hz. A 10 min rest was allowed between stimuli to avoid the myotonic warm-up phenomenon (Rüdel & Senges, 1972). Solutions were changed immediately after recording the response to a stimulus so that the drug contact time was approx. 10 min in each case. Sartorius muscles from frogs, *Littoria aurea*, were used in the same way bathed in Ringer solution (Adrian, 1956). In separate experiments standard intracellular microelectrode methods were used to record action potentials from rat diaphragm muscle fibres in SIF or its low chloride equivalent (SIFH, Bretag, 1973) at 25°C. Responses were obtained to single, 1 ms pulses delivered by fine silver wire extracellular electrodes.

The diuretics tested in this study were frusemide and piretanide (Hoechst), ethacrynic acid (Merck, Sharp and Dohme), mersalyl (Evans), bumetanide (Leo), indapamide (Servier) and acetazolamide (Lederle). For comparison, the known myotonic agents, 2,4-dichlorophenoxyacetic acid (Sigma) and anthracene-9-carboxylic acid (Aldrich), were used. All acids were neutralized with NaOH before injection or addition to bathing solutions. In SIFH, sodium 3,5-diacetamido-2,4,6-triiodobenzoate (Winthrop) was used to replace sodium chloride.

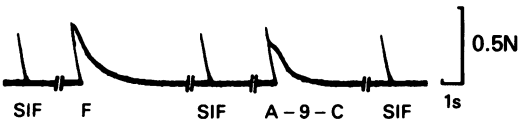

Figure 1 A sequence of isometric tension records from a strip of rat diaphragm muscle stimulated at 10 min intervals in control solution (SIF) and in SIF containing anthracene-9-carboxylic acid, (A-9-C, 22.5 μmol/l) or frusemide (F, 2.5 mmol/l) showing the prolonged contractions typical of myotonia and their reversibility. Resting tension, 0.02N.

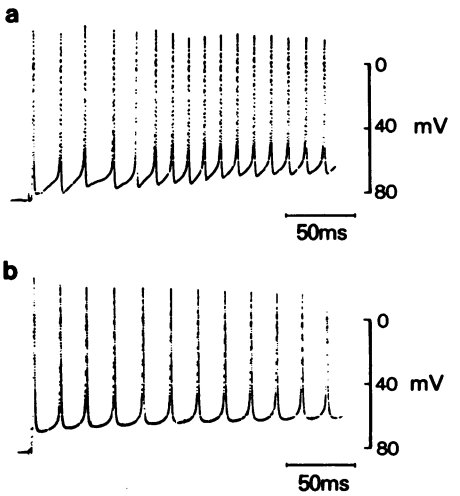

Figure 2 Intracellular microelectrode recordings of myotonic action potentials in rat diaphragm muscle produced in response to a single, 1 ms stimulus via extracellular electrodes. The stimulus artifact may be seen immediately preceding the first action potential of the train. (a) After approximately 15 min bathing in control solution (SIF) containing frusemide 2.25 mmol/l. (b) Another muscle after approximately 15 min bathing in the low chloride solution, SIFH.

Results

Audio EMG responses from normal muscles are characteristically brief, rapid discharges. A few seconds after a bolus injection of frusemide (100 to 250 mg/kg, i.v.), however, myotonic responses could be obtained on insertion of the recording electrode into, or slight movement of the electrode within, leg muscles of the rats. These responses consisted of long trains of discharges of initially increasing and then decreasing frequency: being the equivalent of the 'divebomber' sound as classically recorded from humans and goats with hereditary myotonia (Bryant, 1977). (Actually the firing frequency of the action potential discharges is higher in rat than in human or goat muscle with the result that in myotonic rat muscle the sounds of the audio EMG are more like those of angry bees than of divebombers). The severity of the EMG myotonia was related to the dose of frusemide with the first definite responses occurring at about 100 mg/kg. After the higher doses, myotonic responses were detectable for at least 1 h. Similar trains of discharges could be obtained from rats 30 min after injection with 2,4-dichlorophenoxyacetic acid (200 mg/kg, i.p.) or anthracene-9-carboxylic acid (20 mg/kg, i.p.).

On the isolated muscle preparations, frusemide, ethacrynic acid and mersalyl at concentrations of 2.5 mmol/l all induced greatly prolonged muscle contractions lasting up to several seconds in response to our standard short train electrical stimulus. These myotonic contractions are quite comparable to those produced in the presence of the potent myotonic agent,

Figure 3 As for Figure 1 but showing the effect of acetazolamide (A, 2.5 mmol/l). Myotonia was induced by A in the presence of a subthreshold dose of anthracene-9-carboxylic acid (4.5 μmol/l) (C).

anthracene-9-carboxylic acid (A-9-C), at a concentration of 22.5 μmol/l (Figure 1). At 0.5 mmol/l, frusemide was still capable of inducing significantly prolonged muscle contractions with some effect present down to 0.1 mmol/l. Similar results were obtained with frog sartorius muscle.

Using microelectrodes we recorded after-discharges up to several seconds in duration in these diuretic-treated muscles following a brief electrical stimulus (Figure 2a). These repetitively firing action potentials are the intracellular equivalent of the electrical activity responsible for the EMG 'angry bee' sound. After-discharges and response thresholds determined in this way are indistinguishable from those recorded from the same muscle when bathed in a solution in which a membrane impermeant anion replaces chloride (Figure 2b).

Several other diuretics, bumetanide, piretanide, indapamide and acetazolamide did not show any evidence of directly inducing myotonia *in vitro* at concentrations up to 2.5 mmol/l. However, acetazolamide (2.5 mmol/l) induced myotonia in the presence of 4.5 μmol/l A-9-C, which is a subthreshold dose (Figure 3). The other diuretics all similarly unmasked subthreshold myotonia but unlike acetazolamide their action could not be reproduced in all muscle preparations.

Discussion

Finding diuretic-induced myotonic responses which are indistinguishable from those induced by A-9-C or low chloride solutions has confirmed our suspicion that the diuretics are also myotonic agents: the rapidity of the response *in vivo* precluding electrolyte shifts or metabolic changes as the cause of the myotonia.

Although the minimum dose producing a definite audio EMG response *in vivo* in our rats was 100 mg/kg we feel that a more sophisticated, quantitative EMG assessment (Mrożek, Kamińska & Kwieciński, 1974) could have demonstrated more subtle alterations at much lower doses. The minimum concentration of frusemide producing a noticeable myotonic action *in vitro* was 0.1 mmol/l (33 μg/ml). This concentration could easily be exceeded in the plasma of patients being treated for renal failure where up to 6 g of frusemide (i.v.) per day have been used (Sullivan, Kreisberger & Mittal, 1971): plasma concentrations of 10 μg/ml having been attained with levels above 5 μg/ml being maintained for 24 h following a single oral 500 mg tablet (Tilstone & Fine, 1978).

Reduced extracellular calcium is itself capable of potentiating myotonia (Senges & Rüdel, 1972). It is therefore possible that in the presence of electrolyte alterations such as the decreased plasma calcium and potassium concentrations caused by some diuretics (Dargie & Dollery, 1975), myotonia may be induced by the diuretics at even lower concentrations, perhaps even at normal therapeutic concentrations.

It is now generally agreed that many examples of naturally occurring and chemically induced myotonia result from a lowered membrane chloride conductance (Bryant, 1977). Although we have not measured conductances in the presence of the diuretics, the ability of these agents to block chloride transport in the kidney (Burg & Stoner, 1976), in red blood cells (Motais & Cousin, 1976), and elsewhere (Nicoll, 1978) and their structural relation to myotonic agents which are known to block chloride conductance makes it unlikely that they act on muscle by any other mechanism. This implies that there is a similarity between chloride transport in the thick ascending limb of the loop of Henle in the kidney and in the skeletal muscle membrane. An identity between these transport systems may also be deduced from the known inability of patients with myotonia congenita to concentrate their urine as effectively as normals (Campion & Peter, 1974). Therefore myotonia congenita could well be the result of a generalised disorder of chloride transport having some affinities with Bartter's syndrome, where the defect is postulated to be one of chloride reabsorption in the thick ascending limb of the loop of Henle (Gill & Bartter, 1978). Weakness, cramps, tetany and an inability to concentrate urine are typical features of Bartter's syndrome (Bartter, Pronove, Gill & MacCardle, 1962). The possibility, recently postulated, that there is an 'excess of chloriuretic hormone' in this condition (Grekin, Nicholls & Padfield, 1979), or the equally likely 'lack of an achloriuretic hormone', is intriguing in that such a substance might well also modulate muscle chloride permeability.

Our results suggest that the diuretics and, indeed, any drugs which are monocarboxylic aromatic acids should be considered as potential myotonic agents until proven otherwise. Also esters and other compounds capable of being metabolized to these acids are suspect (Dromgoole, Campion & Peter, 1975). Any such drugs could potentiate pre-existing myotonia in patients or provoke myotonic manifestations in persons with subclinical myotonia. The latter could be as common as one in one hundred in the

population according to Becker's (1979) calculations of the frequency of heterozygotes for recessive generalized myotonia in West Germany. In several documented cases of drug-precipitated myotonia, heterozygotes have been implicated as persons predisposed to myotonia (Becker, 1979).

Several potent diuretics, bumetanide, piretanide and indapamide gave inconsistent results, but certainly seemed to be less myotonic on rat muscle than frusemide, ethacrynic acid and mersalyl acid. However, it is noteworthy that myalgia is a relatively common adverse reaction to bumetanide as it is to frusemide (Cuthbert, 1975) and that muscle stiffness also occurs frequently after bumetanide (Berg, Tromsdal & Widerøe, 1975). Therefore, apart from the acute manifestation of myotonia, the myopathies and neuropathies resulting from chronic exposure to myotonic agents (Rüdiger *et al.*, 1972; Prescott *et al.*, 1979; Wallis, Poznak, Plum, 1970) require consideration. Indeed, the frequent association of dystrophy with myotonia, also in the hereditary myotonias, begs the question of whether there is any casual relationship (Bryant, 1977).

Our demonstration of acetazolamide's ability to potentiate subthreshold myotonia seems to be at variance with reports of its value in the treatment of myotonia (Griggs, Moxley, Riggs & Engel, 1978). Neither acetazolamide nor indapamide are carboxylic acids and differ in this way from the usual myotonic agents. However, Bilezikjian & Bryant (1978) have recently described a non-carboxylic acid compound (SKF 17266) with myotonic potency comparable to that of A-9-C. These considerations and the often severe paraesthesias seen as side effects of acetazolamide therapy (Griggs *et al.*, 1978) make it a doubtful alternative in the treatment of myotonia. On the other hand, its reported antimyotonic activity could reflect an *in vivo* metabolic action or electrolyte redistribution which would be worthy of further study.

Finally, we are concerned that reports of the use of diuresis (Prescott *et al.*, 1979) and in particular frusemide diuresis (Brandt, 1971), in the treatment of herbicide intoxication could encourage the more vigorous application of diuretics in these cases. The common herbicides, being synthetic auxins, are generally monocarboxylic aromatic acids closely related to the diuretics and are known myotonic agents (Bucher, 1946; Dawe & Bretag, 1978). If administered to patients with herbicide intoxication, the myotonic effects of diuretics like frusemide would be expected to add to those of the herbicide with dire consequences. The situation would be compounded by the nephrotoxicity of high doses of herbicide destroying the organic anion secretory system of the proximal convoluted tubule (Koschier & Acara, 1979). The organic anion secretory system is essential to both the excretion of the herbicide and to the diuretic action of frusemide as this is the means by which the latter reaches its site of action in the thick ascending limb of the loop of Henle (Olind, 1979). With neither substance able to be excreted, their combined myotonic actions would predominate. Therefore diuretic drugs which might possibly act on membrane chloride transport should be avoided in treating herbicide intoxication. Forced alkaline diuresis using sodium bicarbonate would appear to be the appropriate treatment (Prescott *et al.*, 1979).

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