Effect of pavetamine on protein synthesis in rat tissue

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ABSTRACT

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Pavetamine, the active principle of plants causing gousiekte in ruminants, was found in this study to be an inhibitor of protein synthesis in the rat heart. Sprague-Dawley rats were injected intra-peritoneally with 8–10 mg/kg pavetamine and the levels of protein synthesis in different organs determined utilizing L-[4-^{3}H]phenylalanine incorporation. In contrast to the more than 23 % inhibition found in heart tissue at 4, 24 and 48 h after administration of pavetamine, the effect on the kidney, liver, spleen, intestine and skeletal muscle was minimal or returned to pretreatment levels within 48 h. These results may offer an explanation for the clinical signs observed in ruminants with gousiekte, where the heart only is affected.

Keywords: Gousiekte, pavetamine, polyamine, protein synthesis inhibition, rats

INTRODUCTION

Gousiekte is rated one of the six most important plant poisonings in southern Africa (Kellerman, Naudé & Fourie 1996), where more than 600 toxic plants are known to occur (Kellerman, Coetzer & Naudé 1988). It is a disease of ruminants characterized by acute heart failure without premonitory signs 6–8 weeks after the initial ingestion of certain rubiaceous plants (Kellerman *et al.* 1988). The active principle contained by plants inducing gousiekte has been isolated (Fourie 1994; Fourie, Erasmus, Schultz & Prozesky 1995) and identified as pavetamine (R. Vleggaar, unpublished data 1997).

The polyamines, to which pavetamine belongs, is a much studied group and contains highly biologically active substances affecting many functions in the body, including cell growth. Pavetamine is the only naturally-occurring member of this group to be incriminated in the poisoning of stock. This study was prompted by the findings that the contractile function of the heart is physiologically impaired (Van der Walt, Van Rooyen, Cilliers, Van Ryssen & Van Aarde 1981) and that pathological changes, especially to myosin, occur in ruminants affected by gousiekte (Schutte, Els, Booyens & Pienaar 1984). The current experiment was undertaken to elucidate the mechanism of action of the active principle in the pathogenesis of gousiekte. Rats were used as experimental model in determining the effect of pavetamine on the rate of protein synthesis.

MATERIALS AND METHODS

Experimental animals and dosing regimen

Young Sprague-Dawley rats (n = 7 x 3) were deprived of food for *c.* 16 h before being injected intra-

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peritoneally (i.p.) at various intervals (*vide infra*) with pavetamine (8–10 mg/kg live mass). Seven control rats per trial, each given 1,5 mℓ saline i.p. instead of pavetamine, were similarly treated. At the end of the experiment, the rats in the three groups (live mass 117–132, 198–209 and 186–213 g, respectively) were anaesthetized by carbon dioxide before cervical dislocation.

Satellite group 1

At the same time a satellite group of rats treated with pavetamine as above (n = 12, 110–208 g live mass) were clinically observed and weighed daily until they died.

Satellite group 2

Another group of control (n = 7, 172–200 g live mass) and treated (n = 13, 172–286 g live mass) rats were euthanazed 4, 5, 6 and 16 days after receiving 6,5–8 mg/kg pavetamine i.p. and examined for macro- and microscopical changes.

Pavetamine extraction

Pavetamine was extracted from *Pavetta harborii* as described by Fourie *et al.* (1995). In each trial, freeze-dried pavetamine was dissolved in distilled water immediately before administration to animals.

Protein synthesis determination

The experimental design was similar to that of Thomson & Wannemacher (1990) and Garlick, McNurlan & Preedy (1980).

In the three trials (*vide supra*) the rate of protein synthesis in rat organs were determined at 4 h, 24 h and 48 h after administration of pavetamine. A flooding dose of 10 µCi L-[4-^{3}H]phenylalanine (Amersham International plc) was administered i.p. to both treated and control animals 2 h before the rats were euthanazed.

Immediately after euthanasia the organs and tissues were perfused *in situ* via the aorta with chilled physiological saline. The heart, liver, kidneys, spleen, intestine and muscle were removed, immediately frozen in liquid nitrogen and stored at –70 °C.

Samples of the various rat organs were analyzed for rate of protein synthesis by measuring the level of radioactivity and protein content. The rate of protein synthesis in tissues can be calculated from the amount of L-[4-^{3}H]phenylalanine incorporated, measured as disintegrations per minute (dpm) per milligram of protein. Tissues were thawed, weighed (*c.* 0.5 g) and homogenized in buffer [0.25 M sucrose, 50 mM HEPES (pH 7.6 with 5 M KOH), 75 mM KCl and 5 mM $MgCl_2$] and made up to a final volume of 10 mℓ using a motor-driven teflon pestle. All samples were kept on ice during the processing procedure. Protein in the samples was precipitated by addition of 1 mℓ of 2.0 N perchloric acid. Samples were centrifuged and the pellets washed twice with 0.2 N perchloric acid. Centrifugation speed (*c.* 600 x *g*) was adjusted to recover precipitated protein quantitatively and to allow uniform resuspension of the pellet with a Vortex mixer. The samples were hydrolyzed by adding 2 mℓ of 1 N potassium hydroxide and warming for 1 h in a water bath at 37 °C. Five hundred microlitres of each sample were added to 4 mℓ of Aquasol and counted by liquid scintillation with a dual-label programme (Packard Tri-Carb 1600CA Liquid scintillation analyzer).

The protein content of each homogenate was measured according to the BIO-RAD spectrophotometric method (Shimadzu UV-visible recording spectrophotometer).

Calculations and statistical analysis

The results for treated rats, measured as L-[4-^{3}H] phenylalanine dpm/mg protein, were plotted as a percentage of the level found for control rats for each tissue at each period.

An analysis of variance (ANOVA) was performed at 4, 24 and 48 h to test for differences between the control and treated animals. The trials were considered as blocked trials as each pair of treated and untreated rats (n = 7) was dissected at the same time (see Table 1). All assumptions of normality and homogeneous variances of treatments for ANOVA were satisfied.

All analyses were performed using the statistical programme GenStat 5 for Windows 2000.

Figures 2 and 3 indicate the pavetamine treatment group computed as a percentage of the control group, while the number of asterisks (*, ** and ***) shows the level of significant difference from the control at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

Pathology

Specimens of organs for light and transmission electron microscopical studies were collected from both the treated and control rats (satellite group 2).

TABLE 1 Skeleton ANOVA

Source of variation	Diff.
BLOCK	6
TMT (between treatments)	1
ERROR (within treatments)	6
Total	13

Light microscopy

From each animal two to three tissue samples of the left ventricular wall, lungs, liver, spleen, small intestine and kidneys were collected in 10% buffered formalin and routinely processed. Sections were stained with haematoxylin and eosin and with Mason's trichrome stain for collagen (Anon. 1968).

Transmission electron microscopy

Specimens from the left ventricular wall were cut in blocks 0.5–1 mm thick and fixed in 2.5% glutaraldehyde at pH 7.2–7.4 for 24 h. Selected blocks were post-fixed in 2% osmium tetroxide for 1 h, dehydrated in a graded ethanol series, passed through propylene oxide as the intermediate solvent, and imbedded in resin (EMBed 812, Set Point Technology Group).

Thick sections were cut for tissue orientation and stained with toluidine blue, while thin sections from selected blocks were stained at room temperature for 20 min in a saturated aqueous solution of uranyl acetate and 3 min in Reynold's lead citrate, with rinsing in between.

RESULTS

The rate of protein synthesis in tissues of control rats was expressed as the amount level of L-[4-^{3}H]phenylalanine incorporated per milligram protein of each tissue (Fig. 1). The rate of phenylalanine incorporation differed between the various tissues examined with the intestine being the highest at 1 179 dpm/mg protein, followed by the kidney, spleen and liver. The heart and muscle had the lowest incorporation rate of 405 and 58 dpm/mg protein respectively.

The results for the treated rats were plotted as a percentage of the control L-[4-^{3}H]phenylalanine dpm/mg protein for each tissue (Fig. 2). Protein synthesis in the heart, liver and kidney at different time intervals after pavetamine administration is compared in Fig. 3.

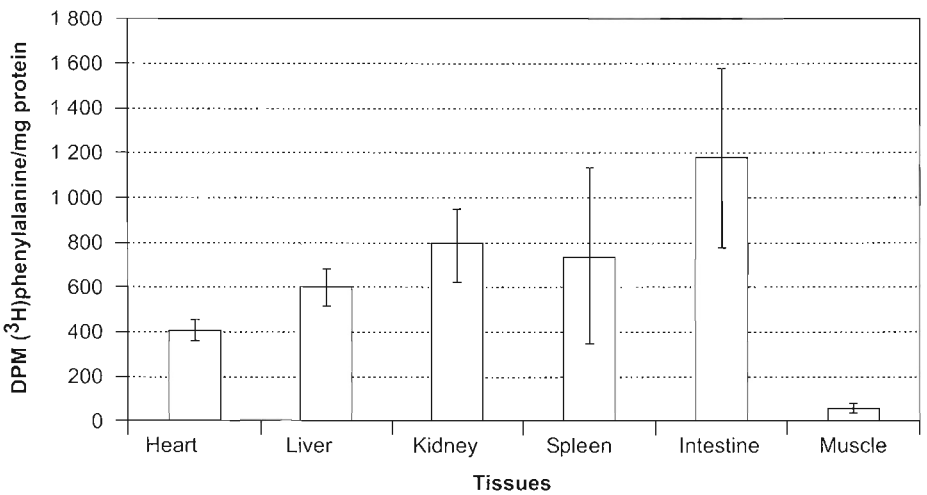

FIG. 1 Protein synthesis in tissues of control rats (n = 7)

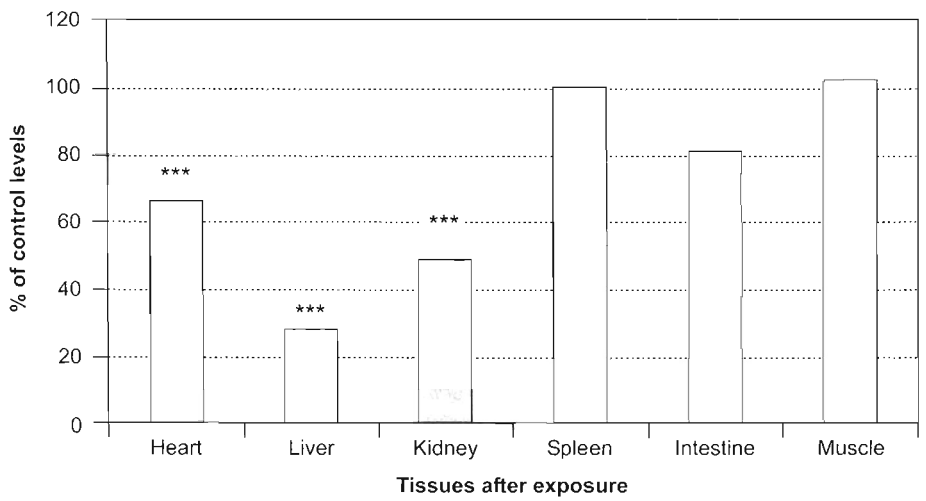

FIG. 2 Protein synthesis in organs of pavetamine-treated rats expressed as a percentage of the controls at 4 h

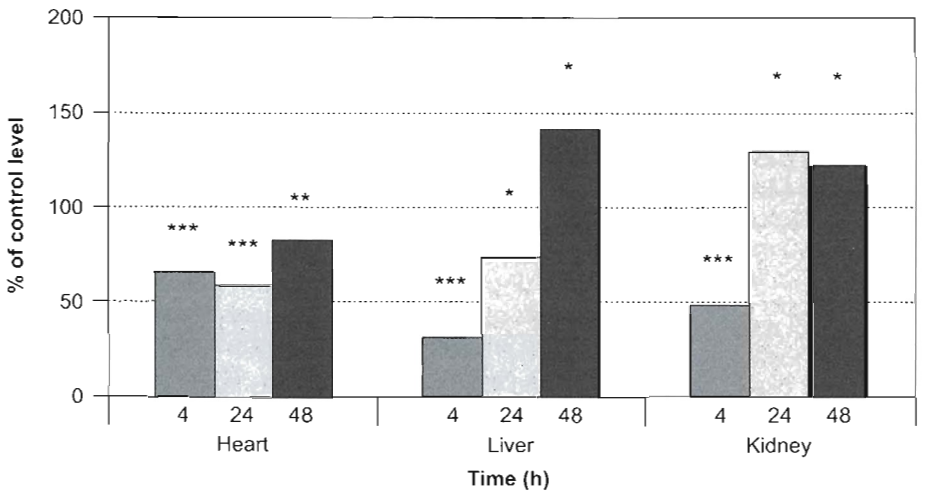

FIG. 3 Effect of pavetamine on the synthesis of protein in rat tissues

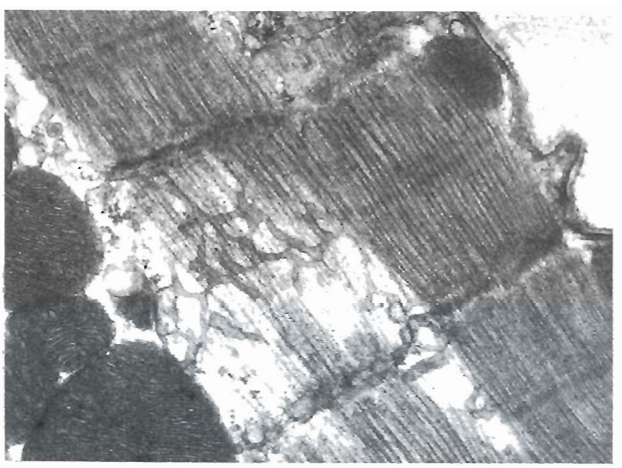

FIG. 4 Focal area of myofibrillar lysis. The characteristic band pattern is replaced by predominantly fine myofilaments intermingled with sarcoplasmic reticulum (X 32 760)

These experiments show that one of the effects of pavetamine is inhibition of protein synthesis which was more pronounced in certain organs than in others (Fig. 2). Protein synthesis levels were less than 66 % in the heart, liver and kidney when compared to control rats at 4 h after exposure to a lethal dose of pavetamine. On the other hand, protein synthesis in muscle, spleen and intestine tissues were hardly affected at this stage. Protein synthesis in the liver and kidneys returned to normal or overcompensated between 24 and 48 h after pavetamine was administered (Fig. 3). In the heart, however, protein synthesis remained suppressed for as long as 48 h after administration.

Satellite group 1

After pavetamine administration no clinical signs were observed until 72 h, when diarrhoea ($n = 2$) and lethargy ($n = 8$) set in. Weight loss of treated rats correlated with decreased feed consumption. The rats died 4 ($n = 2$) to 5 days ($n = 6$) after the treatment.

Satellite group 2

Macroscopical changes included mild to moderate oedema of the lungs with multifocal to coalescent areas of atelectasis in three rats, necropsied 6 days after pavetamine administration. A mild alveolar oedema was confirmed in these three animals when investigated under light microscopy.

Transmission electron microscopy revealeds focal areas of myofibrillar lyses. Affected sarcomeres had a frayed appearance and the characteristic band pattern was replaced by predominantly fine myofilaments intermingled with sarcoplasmic reticulum. The Z-bands were thickened and fragmented (Fig. 4).

DISCUSSION

The rates of protein synthesis in the six tissue types investigated differed,with the skeletal muscle being the lowest and the intestine the highest. Intestinal tissue had a synthesis rate of about twenty-fold and the heart six-fold that of skeletal muscle. The term protein turnover is used to describe the dynamic state of muscle protein which is continuously being synthesized and degraded (Swick & Song 1974; Earl, Laurent, Everett, Bonnin & Sparrow 1978). Earl *et al.* (1978) found that the average turnover rate of muscle protein varies in cardiac and skeletal muscle. In the rat, dog, fowl and mouse turnover in cardiac muscle was more rapid than that in skeletal muscle. In the rat, using [^{3}H]leucine, a ten-fold higher turnover rate than in the *Tensor fasciae latae* was recorded in the ventricles. Swick & Song (1974) reported a six-fold difference between myosin turnover from cardiac and skeletal muscle in the rat. These turnover differences between muscle types can be extrapolated to explain differences in protein synthe-

sis rates found in the current study in the different organs analyzed.

A lethal dose of pavetamine suppressed the protein synthesis in cardiac muscle to below 77 % that of controls, at 4, 24 and 48 h after administration. The rate of protein synthesis in the spleen, intestine and skeletal muscle was unaffected at 4 h after pavetamine administration while in the kidney and liver the rates had returned to pretreatment levels by 24 and 48 h respectively. The effect of pavetamine on protein synthesis in the heart is sustained for at least 48 h, in contrast to the other organs which are either unaffected or where protein synthesis resumes. The contractile proteins in the myocardium are constantly broken down and re-synthesized as part of physiological turnover. Results from the current study in rats enable us to postulate that, while myocardial protein breakdown apparently is unaffected, pavetamine inhibits the synthesis of new myocardial protein. Depending on the half-life of the cardiac protein a point will be reached where breakdown of tissue exceeds synthesis resulting in functional disturbances. Preedy, Smith, Kearny & Sugden (1984) determined that *in vitro* the rate of cardiac protein degradation in rats is 15.7 % per day under physiological workloads. Zak, Ratkitzis & Rabinowitz (1971) concluded that the apparent half-lives of the major cardiac myiofibrillar proteins myosin, actin and tropomyosin in rats are the same (11–12 days). Swick & Song (1974) determined a half-life of myosin from rat ventricle of 4–6 days, using pulse labelling of [^{14}C]aspartate. The half-life of the rat cardiac myosin heavy chain was calculated to be 5–6 days (Martin, Rabinowitz, Blough, Prior & Zak 1977) and 5.5 days by Everett, Prior & Zak (1981). The fact that the rats died 5 days after administration of pavetamine coincides with the half-life quoted for cardiac protein.

No clinical signs were observed in the treated rats during the first few days after administration of the lethal dose of pavetamine. This is a similar pattern to that observed in sheep where after ingestion of plants causing gousiekte the typical period is 4–6 weeks before clinical signs appear—a period about ten-fold longer than in the pavetamine-treated rats. Following intravenous administration of pavetamine to goats a much shorter latent period of 10 days was encountered before death at 15 days (Fourie *et al.* 1995)—a period about three-fold longer than in the pavetamine-treated rats. Inhibition of protein synthesis can thus also explain the typical latent period of gousiekte after ingestion of plants causing gousiekte.

Light microscopical lesions in sheep and cattle exposed to plants inducing gousiekte include cardiac replacement fibrosis, particularly of the subendocardial zone, and myofibre atrophy (Kellerman *et al.* 1988; Prozesky, Fourie, Neser & Nel 1988). A transmission electron microscopical study in sheep showed a reduction in the number of myofilaments, especially myosin (Schutte *et al.* 1984). In the current study in rats there are indications that particularly myosin filaments (Fig. 4) are affected, confirming the results that formation of myocardial protein is inhibited by pavetamine.

Starvation is described in the literature as another factor that influences rates of protein synthesis. Protein synthesis rates in the ventricles of starved rats (15 % weight loss) were found to decrease by up to 50 % after 3 days, compared with fed controls (Preedy *et al.* 1984). In the current study the rate was reduced at 4 h to *c.* 66 % that of control cardiac tissue and weight loss was only 2 % after 24 h, showing weight loss to be an insignificant factor.

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