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THE EFFECTS OF SODIUM PROPIONATE AND ADRENOCORTICOTROPIN ON SERUM CORTISOL LEVELS IN DAIRY COWS

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The effects of adrenocorticotropin (ATCH) and sodium propionate (Na-P) on cortisol concentrations in the blood serum of cows was examined 10 days after calving. A solution of Na-P (1.84 mol/L) was administered as a single dose (1 ml/kg body weight) intravenously to ten cows, while ACTH was given intramuscularly in a single dose of 25 IU per 100 kg body weight to a further ten cows. A control group of cows received physiological saline solution. Cortisol concentrations were determined 8, 30, 60, 120 and 180 minutes after treatment.

It was found that Na-P administration led to a marked increase in serum coristol levels in the examined cows, some animals showing a more marked response than others. The mean maximal increase of nearly seven times the initial value occurred 1 h after administration of Na-P (16.5 ± 2.2 nmol/L to 111.0 ± 24.5 nmol/L). A similar marked increase in serum cortisol levals was noted after ACTH administration (14.2 ± 2.5 nmol/L to 126.0 ± nmol/L), but it occurred after a slight time lag so that the highest serum cortisol concentrations were found after 3h at the and of the experiment. These results show that Na-P has a similar effects on the adrenal cortex as ACTH and its action is very rapid.

Key words: Cows, Na-propionate, ACTH, Cortistol

INTRODUCTION

One of the key roles of glucocorticoids in the organism is stimulation of gluconeogenesis. This action is of special importance in ruminants because more than two thirds of the needs for glucose are satisfied by synthesis from glucogenoplastic substances in these species of mammal (Bergman, 1973). The glucogenic process is particulary marked when the glucose requirement is high such as during pregnancy and lactation. Glucocorticosteroids act by stimulating the supply of precursors from endogenous sources and by activating gluconeogenic enzymes to maximise the utilization of precursors from the diet and body tissues (Ray et al., 1964; Šamanc et al., 1988;).

It has been found that some precursors which are formed by the degradation of carbohydretes in the forestomach can influence the hormonal activity of some endocrine glands to a great degree (Manns and Boda, 1967; Stern et al., 1970; Trenkle, 1970,1978). Peters and Elliott (1984)established that intravenous administration of propionate to cows led to a marked increase in insulin and glucagon concentrations, which confirmed the results of Bartoš and coworkers (1970) in goats.

Since propionate is the main percursor for synthesis of glucose in the liver of ruminants, propionate loading is used as a test for liver functions (Gröhn, 1985). After intravenous administration of propionate there is an increase in blood glucose concentration, on the basis of which the functional state of the liver is assessed. Since the proces of gluconeogenesis is largely dependent on the stimulative influence of glucocorticoids, it was decidet to investigate the possible effect of propionate on cortisol production by the adrenal cortex, because cortisol is the main hormone secreted by the zona fasciculata of this endocrine gland.

MATERIAL AND METHODS

Animals and sample collection. A total of 25 cows of the Holstein breed were included in the investigation. The chosen animals were clinically healthy and no ketone bodies were detected in their urine. Ten days after calving, ten cows of the first experimental group received a solution of sodium propionate (Na-P; 1.84 mol/L) intravenously at the dose of 1 ml/kg body weight. The injection was given at 9 am after milking and feeding. At the same time ten cows in the second experimental group received adrenocorticotropin (ACTH; Galenika, Beograd) intramusculary at the dose of 25 IU/100 kg body weight. The remaining five cows which served as the control group were given 10 ml of physiological saline solution intramuscularly.

Blood samples were taken from the jugular vein of each cow before treatment and then at 8, 30, 60, 120 and 180 minutes after administration of the injections. The serum was separated and stored at -20° C until analysed.

Hormone assay. Serum concentrations of cortisol were determined by radioimmunoassay using a commercially available kit (INEP, Zemun) and following the procedure recommended by the manufacturer.

Statistical analysis. Descriptive statistical parameters were calculated and the statistical significance of differences was determined by two-factor analysis of variance, Friedman's two-way test and the t-test for small samples.

RESULTS

The results obtained for cortisol concentrations in the blood serum of cows before and after administration of Na-P or ACTH are shown in Table 1. In comparison with the control group of cows, it can be seen that there was a marked and rapid increase in serum cortisol concentrations in both experimental groups which was statistically higly significant ($F = 14.00$; $P<.001$). The

increase occurred earlier in the group of cows treated with Na-P, so that already at 8 minutes after injection serum levels of cortisol were significantly higher than in the control group of cows. This may be related to the nature of the stimulating substance and/or the route of administration. After reaching maximal concentrations at 60 minutes after injection, serum cortisol levels decreased in the Na-P treated cows. Following the initial delay, the mean rate of increase of serum cortisol in the group of cows treated with ACTH was similar to the mean rate of increase in the Na-P treated cows, but the increase continued until the end of the experiment (180 min.).

Table 1. Cortisol concentrations (n mol/l) in the blood serum of cows injected with saline (control), propionate or ACTH.

Time		GROUP		
		Control (n = 5)	**Propionate (n = 10)**	**ACTH (n = 10)**
		$\bar{X} \pm SE$	$\bar{X} \pm SE$	$\bar{X} \pm SE$
Initial (0)		11.1 ± 1.9	16.5 ± 2.2	14.2 ± 2.5
After treatment (min)	8	14.2 ±2.5	46.8 ± 7.2	17.0 ± 2.8
	30	17.0 ± 3.6	80.7 ± 12.0	47.6 ± 9.0
	60	20.8 ± 3.2	111.8 ± 24.5	87.9 ± 14.0
	120	20.9 ± 5.0	109.4 ± 36.9	112.8 ± 18.3
	180	14.9 ± 1.8	42.1 ± 11.9	126.2 ± 18.6
Analysis of variance	Group	$F = 14.00 ; p < 0.001$		
	Time	$F = 10.75 ; p < 0.001$		
Tukey – Snedecor D = 22.3				

Table 2. Cortisol concentrations (n mol/l) in the blood serum of two subgroups of propionate treated cows

Time		Subgroup		P
		P1 (n = 5)	**P2** (n = 5)	
		$\bar{X}$ + SE	$\bar{X}$ + SE	
Initial (0)		20.8 ± 2.4	12.1 ± 2.6	< 0.05
After treatment (min)	8	65.4 ± 6.4	28.1 ± 4.6	
	30	113.3 ± 8.2	48.2 ± 7.5	
	60	172.8 ± 28.5	50.7 ± 5.6	< 0.001
	120	200.0 ± 45.0	18.8 ± 2.8	
	180	72.7 ± 12.8	11.5 ± 2.1	
Analysis of variance	Group	$F = 69.3 ; p < 0.001$		
	Time	$F = 11.1 ; p < 0.001$		
Tukey – Snedecor D = 19.2				

Analysis of changes in cortisol concentrations in individual cows indicated that there two types of reaction to Na-P treatment. Thus, five cows reacted with a considerable rise in cortisol concentrations which continued up to 120 minutes after treatment when maximal concentrations of 200 ± 45 nmol/L were attained (Table 2). The remaining five cows had significantly lower initial concentrations

of cortisol ($P<0.05$). The response to Na-P injections was immediate but of milder intensity and shorter duration so that the mean maximal concentration of only 50 ± 6 nmol/L at 60 minutes was followed by a decrease to initial levels at 180 minutes after injection. The differences between the subgroup were statistically highly significant at each time interval after injection of Na-P ($P<0.001$).

DISCUSSION

Many investigators have shown that short chain fatty acids have an important role in regulation of the activity of cells of the endocrine pancreas in ruminants (Manns and Boda, 1967; Bartoš et al., 1970; Stern et al., 1970; Trenkle, 1970, 1978; Istasse and Orskov, 1984; Mineo et al.,1990). Thus, intravenous injections of butyrate into adult sheep induced immediate (5 minutes) increases in plasma concentrations of glucose, insulin and glucagon (Mineo et al., 1990). Having in mind that under physiological conditions butyrate is largely metabolised in the rumen wall and liver, its concentration in peripheral serum is usually low. Therefore, it may be supposed that the other short chain fatty acids produced by the degradation of dietary carbohydrate in the rumen would have a greater effect on the endocrine system. As one of these products, propionate represents an important direct source for glucose synthesis in the liver. Moreover, in cattle and sheep it has been shown that intravenous application of propionate leads to marked changes in the serum concentrations of hormones secreted by the endocrine pancreas (Johnson et al., 1980; Peters and Elliott, 1984). The first rise in serum insulin concentration followed increasing serum levels of propionate within 10 minutes in goats and was more rapid than the reaction to butyrate or glucose infusion (Bartoš et al., 1970).

However, in the available literature no data were found about the possible effect of propionate on serum cortisol concentrations in ruminants. The results of these investigations show that intravenous injection of propionate (1.84 mmol/kg body weight) is followed by a significant increase in cortisol levels in the serum of dairy cows. It should be mentioned that the initial concentrations of cortisol varied widely between individual animals (coefficient of variation (CV) — 42%) and that these differences were magnified after Na-P administration. The greatest differences between individual animals occurred 20 minutes after Na-P injection (CV — 106%). On the other hand the CV within the group of cows receivung ACTH remained constant at about 50% at each examined time interval (e.g. initial CV — 56%; CV at 120 min. — 51%).

Moreover, Šamanc and coworkers (1986) showed that administration of ACTH to ketotic cows reduced the range of individual differences. Thus, the ratio between the greatest and smallest values was 1:6 before treatment, but fell to 1:1.54 hours after giving ACTH. The differences had reappeared 24 h later exhibiting a ratio of similar magnitude (1:8) as the initial ratio.

In the present experiment the response to ACTH was slower than the response to Na-P which may have been partly due to the difference in route of administration but also to differences in mechanisms of action of the examined

agents. Thus, it was found that there were two types of reaction to Na-P administration. Those cows with higher initial levels of serum cortisol reacted by increasing cortisol concentrations many fold within 60 to 120 minutes, whereas the increase in the cows with lower initial cortisol concentrantions was much lower and of shorter duration. This may indicate the possibility of differences in the capacity of the adrenal cortex to react to Na-P. Although the results presented in this paper cover a wide range and were obtained in a relatively small number of animals, they show that propionate affects the activity of the adrenal cortex and rapidly increases serum cortisol concentrations. This is probably a direct effect because increases in blood cortisol following the hypoglycemia induced by insulin infusion occurred after a lag phase of about 30 minutes in lactating sheep (Bassett, 1989). Moreover Na-P infusion leads to hyperglycemia rather than hypoglycemia (Gröhn, 1985). These preliminary investigations indicate that two subpopulations of cows may exist concerning the sensitivity of the adrenal glands to Na-P. Checking and confirmation of these suggestions and the elucidation of the mechanisms involved will be the subject of our further investigations.

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UTICAJ Na—PROPIONATA I ACTH NA KONCENTRACIJU KORTIZOLA U KRVNOM SERUMU MLEČNIH KRAVA

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SADRŽAJ

U radu je proučavan uticaj Na-propionata i ACTH na koncentraciju kortizola u krvnom serumu krava 10 dana posle telenja. Rastvor Na-propionata (1,84 mol/L) u količini od 1 ml/kg telesne mase je davan jednokratno i.v. grupi od 10 krava, dok je ACTH davan i.m. u dozi od 25 I.U./100 kg telesne mase drugoj grupi od 10 krava. Kontrolna grupa krava je dobila fiziološki rastvor i.m. Koncentracije kortizola su određivane u krvnom serumu 8, 30, 60, 120 i 180 minuta posle primene preparata.

Utvrđeno je da i.v. ubrizgavanje Na-propionata izaziva značajno povećanje nivoa kortizola u krvi ispitivanih životinja. Jedan čas posle ubrizgavanja preparata nađeno je povećanje nivoa kortizola i do 7 puta u odnosu na početne vrednosti ($\bar{x}$ = 16,5 $\pm$ 2,2 nmol/L; $\bar{x}$ = 111,0 $\pm$ 24,5 nmol/L). Davanje preparata ACTH izaziva značajno povećanje nivoa kortizola u ispitivanim vremenskim intervalima. Za razliku od Na-propionata najveće povećanje nivoa kortizola u odnosu na početne vrednosti je utvrđeno 3 časa kasnije od davanja preparata ACTH ($\bar{x}$ = 14,2 $\pm$ 2,5 nmol/L; $\bar{x}$ = 126,2 $\pm$ 18.6 nmol/L). Dobijeni rezultati ukazuju da Na-propionat ima sličan efekat na koru nadbubrega kao i ACTH i da se taj efekat u odnosu na ACTH dosta ranije ispoljava.