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BIPHASIC PROLACTIN RELEASE BY HALOPERIDOL AND PERPHENAZINE IN LACTATING AND PREGNANT COWS AND EWES

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Abstract—Single i.m. injections of haloperidol or perphenazine to lactating or non-lactating (pregnant) cows and ewes resulted in bi- or multiphasic elevation of serum prolactin in most of the treated animals, as determined by radioimmunoassay (RIA) up to 16 days after treatment. An immediate rise in serum prolactin occurred 1–4 hr after administration of the drugs, with perphenazine yielding a higher and sharper increase than haloperidol, especially in the non-lactating animals. Sixty-five per cent of the haloperidol-treated, but only 21 per cent of the perphenazine-treated animals, exhibited a second prolactin peak within 3–9 days of treatment, while 15 per cent and 0 per cent, respectively, showed a third peak at an even later stage. These results demonstrate that exhaustion of pituitary prolactin is more profound after perphenazine than following haloperidol, and that the ratios of the first peaks of prolactin to the corresponding basal concentrations are higher in non-lactating than in lactating cows and ewes.

Ever since neuroleptics have been used in an attempt to increase milk yield in lactating animals, the possibility of prolonging their lactation periods, rather than causing short but high levels of prolactin, was investigated [1]. Phenothiazines and reserpine were studied at first, for their biphasic effect on the release of epinephrine [2] which, in turn, was established as a prolactin-release inhibitor [3]. Later, Arai and Suzuki [4], as well as McNeilly and Lamming [5], demonstrated a biphasic effect of reserpine and perphenazine on the lactogenic response in male rats and on prolactin levels in non-lactating sheep, respectively.

As the Israeli–Friesian cows and Awassi ewes are renowned for their high milk yield, we decided to compare the effect of two neuroleptics, haloperidol and perphenazine, on the prolactin concentrations in these animals during lactation. This study was extended over a relatively long period (16 days) after a single i.m. injection, and the serum prolactin concentrations in the lactating cows and ewes were compared to the corresponding values in non-lactating ones. The current study aims to show whether neuroleptics evoke a multipeak increase in prolactin concentrations, and whether such elevations are of significance for lactation or pregnancy.

METHODS

Twenty Israeli–Friesian cows (460–675 kg b.wt.) and 25 fat-tail Awassi ewes (50–70 kg b.wt.) were used for this experiment. Nine of the cows and 12 of the ewes were lactating, yielding 10–26 and 0.7–2.4 liters of milk daily, respectively; the remaining animals were non-lactating and in their late pregnancy. The animals were housed outdoors and fed grass silage and pelleted ration-containing cereals. All animals were accustomed to handling several days before the experiment including blood sampling, thus reducing possible prolactin surges due to stress.

Treatment. Haloperidol was dissolved in absolute ethyl alcohol, diluted 1:1 with normal saline and injected i.m. into 4 lactating cows, 4 lactating ewes, 6 non-lactating (pregnant) cows and 6 non-lactating (pregnant) ewes, at a dose of 5 mg/0.1 ml/kg b.wt. Perphenazine was dissolved in minimal amounts of 0.3M HC1, diluted with normal saline to yield the same concentration and the same dose injected into 4 lactating cows, 5 lactating ewes, 4 non-lactating cows and 6 non-lactating ewes. The additional 6 animals served as controls and were injected with 0.1 ml/kg 50% ethanol or 0.03M HCl. The drugs or the solvents were injected into the lactating animals one hr after the morning milking, and into the non-lactating animals between 7–9 a.m. All experiments were carried out simultaneously within a 3-week period, during the summer.

Prolactin RIA. Blood was sampled for RIA from the jugular vein before treatment, and 30 min, 1, 2, 3, 4, 6, 8 and 12 hr following treatment. Further samples were taken once daily for the next 16 days within 1 hr of the morning milking, centrifuged within 2 hr of sampling and their sera kept at −20° pending assay.

Prolactin was assayed by the double-antibody RIA technique, as modified from Arai and Lee [6]. Ovine prolactin NIH–P–S9 (27 i.u./mg) and bovine prolactin NIH–P–B1 (13.0 i.u./mg) served for the antibody production, radioiodination with ^{125}I and as standards. Antisera to both ovine and bovine prolactins were raised in rabbits and were used as the second antibody. All assays were performed in 2–3 dilutions and computed as ng/ml serum.

As every animal served as its own control, there was no need for comparing treatments statistically, except for calculating the mean ± S.E.M. of the peak-to-peak ratio. The increase of serum prolactin in the control animals was negligible. We therefore chose to express the height of the first prolactin peak as the ratio between its first high level and its pretreatment ('basal') concentration.

Table 1. Ratios between serum prolactin concentrations at peak time after treatment with haloperidol or perphenazine and serum prolactin concentrations before treatment ('basal' concentrations)

	Haloperidol i.m. treatment		Perphenazine i.m. treatment	
Animal	Ratios of first peak to basal concentration	Second or third peak/ basal prolactin concentration	Ratios of first peak to basal concentration	Second or third peak/ basal prolactin concentration
Lactating cow	2.75	2.39	35.00	*
Lactating cow	2.12	1.39	18.00	*
Lactating cow	19.93	*	19.35	*
Lactating cow	3.19	3.07	33.60	*
Mean ± S.E.M.	7.00 ± 8.63	2.28 ± 0.84	26.49 ± 9.06	—
Lactating ewe	7.43	*	57.19	*
Lactating ewe	5.22	*	58.04	*
Lactating ewe	7.73	*	40.36	*
Lactating ewe	6.09	*	29.33	*
Lactating ewe	—	—	53.11	*
Mean ± S.E.M.	6.62 ± 1.17	—	47.59 ± 12.41	—
Non-lactating cow	1.21	2.62	16,460.00	*
Non-lactating cow	3.23	1.71	25,300.00	1961.67
Non-lactating cow	3.26	11.77	38,635.00	*
Non-lactating cow	2.36	7.41	2,452.00	276.40
Non-lactating cow	3.39	16.71	—	—
Non-lactating cow	1.21	3.95	—	—
Mean ± S.E.M.	2.43 ± 1.01	7.36 ± 5.88	20,711 ± 7,603	
Non-lactating ewe	1.11	1.04	1,118.18	*
Non-lactating ewe	1.16	1.80	600.89	*
Non-lactating ewe	3.26	3.38	61.42	53.53
Non-lactating ewe	2.66	7.18	30.46	395.15
Non-lactating ewe	3.59	3.33	973.43	*
Non-lactating ewe	1.09	*	18.13	*
Mean ± S.E.M.	2.15 ± 1.16	2.75 ± 1.28	417.08 ± 204	

* Blood was sampled up to 16 days after injection without observing a second peak.

RESULTS

Haloperidol caused an immediate and marked rise in the serum prolactin concentration in all 8 lactating and in 8 of the 12 non-lactating pregnant animals that had received the drug. Perphenazine induced an even higher prolactin release in all 9 lactating and in 9 of the 10 non-lactating cows and ewes. Thirteen of the 20 animals treated with haloperidol, but only 4 of the 19 perphenazine-treated animals showed a second rise in serum prolactin concentration. A third elevation occurred in 3 of the haloperidol-treated, but in none of the perphenazine-treated animals. The mean (± S.E.M.) ratios between serum prolactin concentrations at peak time after treatment and serum prolactin before treatment are tabulated in Table 1.

Never were there any two prolactin peaks on the same day: the second peak was obtained 3–9 days after the start of the treatment, and the third peak appeared towards the beginning of the third week, around day 12–16. There was no significant change in milk yield during the entire experimental period.

DISCUSSION

In previous experiments the basal serum prolactin concentrations in lactating and pregnant Israeli–Friesian cows and ewes were measured [7]. The results have shown that haloperidol causes a rapid rise in serum prolactin concentrations in lactating ewes, which drop to pre-administration values within 10 hr of treatment [8]. It was further shown that injections of perphenazine, which stimulated mammary development in dairy ewes, did not increase their milk yield [9]. It was assumed that this was due to the short-lived elevations in prolactin during the treatment. As these were short-term experiments (1–2 days each), and the biological half-life of phenothiazines is long [10], it was impossible to register eventual second and third peaks. A similar difficulty—of a missed peak—is implied in the work by McNeilly and Lamming [5], who collected blood samples from non-lactating sheep 10 hr–8 days after perphenazine administration, and where the first peak was undoubtedly missed.

In two previous experiments, single injections of tritiated perphenazine [11] and tritiated haloperidol [12] caused a dual elevation of prolactin (biphasic effect) in some of the animals. The number of animals that responded by double peaks was smaller than in the present experiment. This can be explained by the shorter experimental period (5–8 days) of the previous study, which would have prevented the third peak from being measured.

In their histological work, Arai and Suzuki [4] observed that after a single injection of reserpine the first changes appear four days later, followed by a second prolonged phase that covers days 5–14. Accordingly, more of our animals might have demonstrated a third peak, if the experimental period had been extended to more than 16 days. The drawback in Arai and Suzuki's

findings is that, in general, histological observations are difficult to assess as they are the result of a cumulative effect of a number of hormones on the lobuloalveolar system, from which the effect of a single factor, such as prolactin, can not be isolated. Moreover, in similar experiments from this laboratory [13], 5 mg/kg daily perphenazine injections into oestradiol-primed virgin rats exhibited a first mammotrophic peak around the first week, followed by additional peaks during the 10 weeks of the treatment. Higher perphenazine doses advanced the first peak to the second day, but the rats were too depressed to survive a second peak.

Our results on the exhaustion of the pituitary after both neuroleptics are in agreement with those of Grosnevor *et al.* [14], who showed that exogenous prolactin increased the amount of endogenous prolactin accumulation by the pituitary of lactating rats, and that such an effect is already monitored several hours after the injection. It is obvious from the present results that the exhaustion of the pituitary is much more profound after perphenazine than following haloperidol administration, where reaccumulation of the hormone is therefore somewhat retarded. It also seems that the prolactin elevations obtained in the current study are higher than the circadian peaks of circulating prolactin.

With regards to the method of comparing the peak height to the pretreatment ('basal') concentrations, it was already suggested by Gugen *et al.* [15] that the term 'basal prolactin concentration' is not of much value in itself, as this might vary in a relatively wide range within the same group of subjects. Therefore, the peaks in our studies were compared to the pretreatment hormone values. In this way each animal served as its own control, and thus enabled comparison between absolute values of different experimental groups.

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