Abstract

Clonidine is a specific $\alpha$-2-adrenoreceptor agonist that stimulates growth hormone (GH) release in animals and humans. This drug was used to study the GH and prolactin (PRL) secretory response in dairy cows and heifers. An i.v. infusion of 10 $\mu$g/kg body weight induced GH release to a peak concentration after 30–60 min, while 2 $\mu$g/kg had no effect on GH secretory patterns. Plasma PRL decreased significantly ($P<0.01$) starting 15–60 min after both doses of clonidine, this effect lasting up to 6 h. Clonidine significantly lowered plasma insulin ($P<0.01$) and raised plasma glucose ($P<0.01$). The changes in plasma GH, PRL, insulin and glucose differed significantly between doses, the 10 $\mu$g/kg dose being more effective ($P<0.01$). The results of our investigation in dairy cattle provide evidence of (i) an increase in GH release after 10 $\mu$g/kg clonidine; (ii) a concomitant decrease in PRL secretion, hence GH and PRL secretion in cattle appear inversely controlled; (iii) a significant difference between the effects of the 2 and 10 $\mu$g/kg doses and (iv) no relationship between the changes in plasma GH and PRL after clonidine and plasma hormone levels before treatment.


Introduction

Much knowledge has been gained in the past few years as to how neuroactive drugs influence brain neurotransmitter synthesis or action. Clonidine, a member of this class, is a specific $\alpha$-2-adrenoreceptor agonist that elicits growth hormone (GH) release in the rat (Durand et al. 1977, Siever et al. 1987), cat, dog, monkey (Muller 1987), sheep (Thomas et al. 1994) and man (Lal et al. 1975) through stimulation of endogenous GH-releasing hormone (Muller 1987).

Clonidine appears not to be involved in the regulation of prolactin (PRL) secretion in man and rat, or of adrenocorticotropic hormone, follicle-stimulating hormone, luteinizing hormone or thyroid-stimulating hormone secretion (Lal et al. 1975). It does, however, appear to induce a rapid and significant reduction in serum PRL levels in non-human primates (Gold et al. 1978). In heifers, clonidine elicits a significant GH secretory burst at doses ranging from 5 to 30 $\mu$g/kg body weight (Gorewit 1981, Kuehner et al. 1993) while pituitary PRL secretion was not affected by 20 $\mu$g/kg but was acutely stimulated by 2 $\mu$g/kg (Gorewit 1981). These findings imply a dose-dependent $\alpha$-2-adrenergic secretory dependence for bovine PRL. In man, studies with apomorphine, a selective dopamine receptor agonist, suggest an inverse dopaminergic system control of GH and PRL secretion, and central $\alpha$-adrenergic clonidine stimulation has been confirmed (Lal et al. 1973).

The present study, therefore, investigated the pituitary response to clonidine in dairy cattle. The specific objective was to analyse the plasma GH and PRL secretory patterns after intravenous clonidine. We also measured blood glucose and insulin to assess the influence of clonidine on centrally and peripherally regulated autonomic functions.

Materials and Methods

Animals and experimental design

Twelve Holstein Friesian cows, 4–5 years old, in late lactation but not pregnant, and four Holstein Friesian heifers, 10 months old, were used. The animals were divided into three groups; group I consisted of nine cows, group II of three cows and group III of the four heifers. To minimise stress the animals were implanted with an indwelling catheter in the jugular vein (Nutricath, Vygon, Ecouen, France). On the day after cannulation, the animals were infused (i.v.) over 5 min with 10 ml 0.9% saline solution, and with clonidine (Catapresan; Boehringer Ingelheim, Italy) dissolved in 10 ml 0.9% saline, the day after. The cows of group I were given...
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10 µg/kg body weight (bw), and the cows of group II and the heifers of group III were given 2 µg/kg bw. All the experiments started at 1400 h. Seven animals of group I were tested in June, while the other two animals of group I and those of groups II and III were subjected to experimentation in September.

During both treatments, with saline or clonidine, blood samples were drawn into heparinized tubes starting 30 min before infusion, then at −15, 0, 15, 30, 60, 90, 120, 150 and 180 min. Two further samples were taken at 6 and 23 h. The plasma obtained after centrifugation at 2500 r.p.m. for 10 min was divided and stored at −40 °C until assayed.

For analysis of the plasma patterns of the examined parameters three periods were considered: (1) pretreatment period: −30, −15 and 0 min before clonidine and saline infusion; for each animal the mean of these points was considered as the basal level; (2) control-treatment period: from 15 min to 23 h after saline infusion; (3) clonidine-treatment period: from 15 min to 23 h after clonidine infusion.

Hormone assays

Bovine GH (bGH) and PRL (bPRL) concentrations were determined using enzyme immunoassays previously described (Secchi et al. 1988, 1991). The sensitivity was 0·25 ng/ml for bGH and 0·1 ng/ml for bPRL. The intra-and inter-assay coefficients of variation ranged between 2·6–5·1% and 8·5–12·7% respectively for bGH, and 3·0–7·1% and 6·0–14·5% respectively for bPRL.

Plasma insulin concentrations were determined by double-antibody radioimmunoassay. The detection limit was <2 µU/ml and the cross-reactivity with bovine insulin was 100% (Pharmacia Insulin RIA, Kabi Pharmacia Diagnostics AB, Uppsala, Sweden).

Plasma glucose

The glucose content of each plasma sample was determined by the glucose oxidase enzymatic method (Glucose GOD-Perid, Boehringer Mannheim, Mannheim, Germany).

**Figure 1.** bGH plasma patterns (means ± s.e.m.) after clonidine or saline. (a) group I: nine cows treated with 10 µg/kg clonidine; (b) group II: three cows treated with 2 µg/kg; (c) group III: four heifers treated with 2 µg/kg. The vertical line indicates the time of treatment. Control and treatment patterns are significantly different in group I (F=6·70; P<0·01) and not different in groups II and III (F=1·50; P>0·1). Asterisks mark points outside the 99% confidence limits and significantly different from the corresponding control values (group I: t=5·93; P<0·01).
A preliminary analysis of the GH, PRL, insulin and glucose plasma concentrations in the three groups showed: (i) a high correlation between mean plasma concentrations and standard deviations within each group and (ii) a significant difference between mean plasma concentrations of each animal. For these reasons a logarithmic transformation of all data was carried out so that a good degree of homoscedasticity was achieved and all values were corrected for the differences between individual plasma concentration means.

Since values during the pre-treatment and post-control treatment periods showed no significant autocorrelation, the subsequent observations were considered as independent and all data were analysed by regular analysis of variance (ANOVA). Means for pre-treatment and post-control treatment periods showed no significant differences at different times, so a control chart was set up where the central line is the overall mean of the control observations (point 0 on the ordinate axis). The upper and lower control limits were calculated at a distance of ±\( \sigma / \sqrt{n} \) from the central line, with Student’s \( t \) set at the 99% confidence level. Thus the upper and lower control limits were the confidence limits of the mean. The log-transformed mean values of the post-clonidine treatment period were plotted on the same control chart. Points falling outside the control limits indicated a possible significant influence of clonidine. For the times for which the means of the post-clonidine treatment pattern lay outside the control limits, the general linear hypothesis was tested, \( H_0: \mu_t=\mu_0 \) where \( \mu_t \) was the vector of post-clonidine treatment means outside the control limits and \( \mu_0 \) the control means for the corresponding period. The significance of differences between clonidine and saline treatments at any given time was established by Student’s \( t \)-test.

The 10 µg/kg and the 2 µg/kg clonidine treatments were compared by applying the general linear hypothesis. In this case, only the means outside the control limits for both treatments at the same time were considered.

The maximum GH plasma concentration observed and the time of its occurrence were obtained from individual data. The bPRL and insulin mean decreases and the
glucose increase were calculated for each animal and were the mean of data not within the confidence limits. The bGH and glucose increases and the bPRL and insulin decreases were calculated as percentage of the basal levels.

Computer analyses were carried out using the Gauss program, version 2.2 (Aptech Systems Inc., Kent, WA, USA).

Results

Plasma bGH

The bGH secretory patterns of groups I, II and III are shown in Fig. 1 (means ± s.e.m.). Cows in group I showed a highly significant difference in plasma bGH after 10 µg/kg clonidine (P<0.01). The mean bGH pattern was outside the 99% confidence limits at 30 and 60 min (lower and upper limits ± 7.02) and bGH levels measured during this peak were significantly higher than the corresponding control values (P<0.01). The GH increase varied from 59% to 212% (mean 120.9 ± 18.8; n=9) and was unrelated to the individual basal levels.

Cows in group II and heifers in group III showed no significant changes in plasma bGH after 2 µg/kg (P>0.1) and no hormonal concentrations were significantly outside the corresponding confidence limits (group II lower and upper limits ± 13.62; group III: ± 12.80).

Statistical analysis of pre-treatment and post-control treatment periods for the three groups showed that neither blood sampling nor saline infusion had any effect on plasma bGH (P>0.1).

Plasma bPRL

Fig. 2 shows the bPRL secretory patterns of groups I, II and III (means ± s.e.m.). Both 10 and 2 µg/kg clonidine significantly influenced plasma bPRL (P<0.01). The mean bPRL pattern of cows in group I fell outside the 99% confidence limits (lower and upper limits ± 4.63) from 15 min to 6 h after 10 µg/kg clonidine; bPRL at these times was significantly lower than the corresponding control values (P<0.01). The decrease ranged from 49% to 86% (mean 68.4 ± 4.6; n=9) and was independent of the basal level.

**FIGURE 3.** Plasma insulin patterns (means ± s.e.m.) after clonidine or saline. (a) group I: nine cows treated with 10 µg/kg clonidine; (b) group II: three cows treated with 2 µg/kg; (c) group III: four heifers treated with 2 µg/kg. The vertical line indicates the time of treatment. Control and treatment patterns of all the groups are significantly different (group I: F=17.06; group II: F=7.33; group III: F=51.83; P<0.01). Asterisks mark points outside the 99% confidence limits and significantly different from the corresponding control values (group I: r=14.80; group II: r=6.30; group III: r=19.55, P<0.01).
The mean bPRL pattern of cows in group II was below the lower confidence limit (lower and upper limits ±17.27) from 15 min to 180 min after clonidine; during this period hormonal levels were significantly lower than the control values ($P<0.01$). The decrease ranged from 36% to 78% (mean 62.1 ± 12.8; $n=3$).

Heifers in group III showed bPRL concentrations significantly outside the confidence limits (lower and upper limits ±14.91) from 60 min to 6 h after clonidine ($P<0.01$). The decrease ranged from 33% to 57% (mean 47.7 ± 4.1; $n=4$).

In cows there was a significant difference in the effects of the two doses of clonidine ($t=2.68; P<0.01$), the 10 µg/kg dose being more effective.

Analysis of pre-treatment and post-control treatment periods for the three groups showed that blood sampling and saline infusion had no effect on plasma PRL concentrations ($P>0.1$).

**Plasma insulin**

The insulin secretory patterns of the three groups are shown in Fig. 3 (means ± s.e.m.). Clonidine lowered insulin within 15 min after injection in all three groups ($P<0.01$). Plasma insulin remained low for at least 3 h after the 10 µg/kg dose (99% confidence limits: ±8.57) and 2 h after the 2 µg/kg dose (99% confidence limits, group II ±9.85; group III ±8.53); during these periods the plasma concentrations remained significantly below the control value ($P<0.01$).

Neither blood sampling nor saline infusion influenced hormonal plasma concentrations ($P>0.1$). Clonidine at 10 µg/kg had a significantly greater effect on plasma insulin than 2 µg/kg ($t=3.45; P<0.01$).

**Plasma glucose**

Fig. 4 shows the plasma glucose patterns of the three groups (means ± s.e.m.). Clonidine significantly raised plasma glucose in all groups ($P<0.01$), while blood sampling and saline infusion had no effect ($P>0.1$).

The hyperglycemia started at 15 min and peaked 60–180 min after treatment. The animals given 10 µg/kg clonidine still had high plasma glucose after 6 h, by which time the effects of clonidine were not significant.

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**Figures and Captions**

**Figure 4.** Plasma glucose patterns (means ± s.e.m.) after clonidine or saline. (a) Group I: nine cows treated with 10 µg/kg clonidine; (b) group II: three cows treated with 2 µg/kg; (c) group III: four heifers treated with 2 µg/kg. The vertical line indicates the time of treatment. Control and treatment patterns of all the groups are significantly different (group I: $F=46.77$; group II: $F=12.10$; group III: $F=21.95$; $P<0.01$). Asterisks mark points outside the 99% confidence limits and significantly different from the corresponding control values (group I: $t=2.68$; group II: $t=1.15$; group III: $t=15.06$; $P<0.01$).
time all the animals given 2 μg/kg had normal concentrations (99% confidence limits: group I ±3.98; group II ±9.75; group III ±8.48). During these times plasma glucose was significantly higher than the control value (P<0.01).

In cows there was a significant difference between doses of clonidine (t=5.29; P<0.01), the effect being more pronounced after 10 μg/kg.

Discussion

GH and PRL are similar hormones synthesised and secreted by two acidophil cell types of the adenohypophysis (Nilson et al. 1983). Their release is regulated by specific hypophysiotropic peptide hormones of the central nervous system. In addition, neurotransmitters and a number of neuroactive drugs can modulate both the central and the pituitary GH and PRL secretory control mechanisms (Muller 1987, Ben-Jonathan et al. 1989).

The GH releasing effect, together with the lack of noticeable side effects, were the main reasons for using clonidine for pharmacological manipulation of hormonal secretion in the present experiments, in line with the report by Pintor et al. (1987) on the therapy of pubertal children with constitutional growth delay.

Our investigation in dairy cattle provides evidence of (i) an increase in GH release after 10 μg/kg clonidine; (ii) a concomitant decrease in PRL secretion; (iii) a significant difference between the effects of the 2 and 10 μg/kg doses, 10 μg/kg being more effective and (iv) no relationship between the changes in plasma GH and PRL after clonidine treatment and basal levels of plasma hormones.

Basal levels of bGH in dairy cows differed widely between animals. This difference between individual bGH plasma concentrations is a characteristic of the bovine species (Kazmer et al. 1986, Secchi et al. 1988), and it has been suggested that elevated GH concentrations might depend on the genetic selection for milk yield (Kazmer et al. 1986, Lovendahl et al. 1991, Woolliams et al. 1993). Basal levels of bPRL in the bovine are similar for animals in the same season. However concentrations rise significantly during the period May—Aug as previously described for bovine and other species (Curlewis 1992, Borromeo et al. 1994); this explains the difference between the bPRL plasma concentrations of animals subjected to experiments in June or September.

The nine cows given 10 μg/kg bw responded to clonidine with a GH secretory burst smaller than that reported by Kuehner et al. (1993) who found peak values were ten times the basal levels. The present data are more consistent with the findings described by Gorewit (1981), although both these studies used 10-month-old heifers.

In the same study, Gorewit (1981) reported no changes in bPRL concentration after 20 μg/kg clonidine but noted a significant secretory peak, with double the normal levels, after 2 μg/kg. Conversely, in our experiments with lactating cows, 10 μg/kg clonidine lowered the bPRL plasma concentration (mean decrease 68.4%).

In an attempt to clarify this contradiction we treated four 10-month-old heifers as well as three cows with 2 μg/kg clonidine. Under these conditions, clonidine caused no significant bPRL peaks in either heifers or cows and in fact there was a significant drop in bPRL (62.1% in cows, 47.7% in heifers). This decrease was smaller than in the animals given 10 μg/kg and of shorter duration. Therefore in our experiments clonidine appears to inhibit PRL release in a dose-dependent manner.

In our study clonidine lowered plasma insulin and raised plasma glucose in heifers and cows. The percentage increases in plasma glucose after 10 and 2 μg/kg clonidine were greater than in humans (Lal et al. 1975, Hunt et al. 1986). Although the increase in plasma glucose may be largely due to the fall in insulin levels reported in this study, other mechanisms of action of clonidine should also be considered. We report that clonidine, at 10 μg/kg, increases plasma GH. As GH at high doses has been shown to induce hyperglycemia in the bovine (Bourne et al. 1977), a contribution by this hormone to the increased blood glucose levels may be possible. However 2 μg/kg clonidine also causes hyperglycaemia, although there is no stimulation of GH release. Other possible modes of action of clonidine include a direct stimulatory effect on the liver via α-2-adrenergic receptors (Gorewit 1980) to increase glucose output, glycogenolysis and inhibit glucose uptake. At the same time clonidine may exert this effect centrally via activation of the sympathetic nervous system (Goodman & Gilman 1990). Similar direct or indirect effects of clonidine occur at the level of the pancreas to inhibit insulin release (Metz et al. 1978).

In conclusion, this is the first report of an inverse control of GH and PRL secretion in clonidine-stimulated dairy cattle. The data indicate that the bPRL secretory mechanism is more sensitive to clonidine action than bGH, and suggest that the bovine species could be a new experimental model for the studies described above.

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