The effects of medetomidine and its reversal with atipamezole on plasma glucose, cortisol and noradrenaline in cattle and sheep

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In the present study, we report the effect of medetomidine followed by atipamezole on plasma glucose, cortisol and noradrenaline in calves, cows and sheep. Eight calves, eight lactating dairy cows and eight adult female sheep were included in a crossover trial. The animals were injected i.v. with medetomidine (40 μg/kg), followed 60 min later by atipamezole i.v. (200 μg/kg) or saline. The wash-out period between experiments was 1 or 2 weeks. In every animal, medetomidine induced a marked hyperglycaemia, which was reversed by atipamezole. Cortisol levels increased significantly in cows and sheep, reaching levels 4–8-fold higher than the baseline levels 25–45 min after injection of medetomidine. Atipamezole did not affect the cortisol levels, except in sheep where an increase was observed. Plasma levels of noradrenaline decreased in cows and sheep after medetomidine injection, reflecting the inhibition of sympathetic activity by the drug. After injection of the antagonist, there was a large increase in noradrenaline levels. In conclusion, a high dose of medetomidine does not seem to reduce the overall endocrine stress response in cattle and sheep, which has previously been reported in other species.

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INTRODUCTION

Medetomidine produces sedation and analgesia in a wide range of mammalian species and is the most selective α_2-agonist used in veterinary medicine. The sedative, cardiovascular and respiratory effects of medetomidine may be reversed by the highly selective α_2-antagonist atipamezole. This agonist–antagonist combination is currently licenced for the use in dogs and cats, and has also proven useful for the reversible immobilization of non-domestic animals, ruminants included (Jalanka, 1993).

It is recognized that certain environmental or management conditions, termed stressors, to which animals are exposed, induce alterations in the hypothalamic–pituitary–adrenal (HPA) axis and activate the sympathetic nervous system. The secretion of adrenocorticotrophic hormone (ACTH), the pituitary hormone controlling steroidogenesis in the adrenal cortex, is increased, thereby increasing the secretion of cortisol (Dickson, 1993), while stimulation of the sympathetic nervous system increases the production and release of catecholamines from the central and peripheral nervous system. An increase in cortisol or catecholamine levels has, therefore, been regarded as being indicative of stressful conditions (Minton, 1994).

The effect of α_2-agonists on the stress response has been studied in horses and humans (Raekallio *et al.*, 1991, 1992; Scheinin *et al.*, 1998). Raekallio *et al.* (1992) found that the α_2-agonist detomidine reduced the stress response in horses, with catecholamines and cortisol levels being reduced after its injection. Kallio *et al.* (1988) suggested an inhibitory role of α_2-adrenoceptors in the regulation of cortisol secretion, as medetomidine inhibited cortisol secretion in humans.

A hyperglycaemic response to α_2-agonists in various species is well documented. It is caused by stimulation of α_2-adrenoceptors in the β-cells in the endocrine pancreas, resulting in the inhibition of insulin release (Hsu & Hummel, 1981; Abdel El Motal & Sharp, 1985; Osman & Nicholson, 1991).

Reversing the effects of α_2-agonists with selective α_2-antagonists, such as atipamezole, can give rise to side-effects as a result of elevated levels of catecholamines. Antagonism may, therefore, result in cardiac arrythmias and hypertension (Carroll *et al.*, 1997). Clinically, excitement and muscle tremor can be observed, especially if the antagonist is injected i.v. (Jalanka & Röken, 1990; Ranheim *et al.*, 1999, 2000).

To our knowledge, the effect of α_2-agonists subsequentally followed by an α_2-antagonist on plasma cortisol and catecholamine levels, has not previously been reported in ruminants. The effects of atipamezole on the α_2-agonist induced hyperglycaemia were also of interest.

In this study we report the effects of medetomidine and its reversal with atipamezole on plasma concentrations of glucose, cortisol and catecholamines in lactating dairy cows, dairy calves and sheep.

MATERIALS AND METHODS

Animals

The following groups of animals were included in the study:

Calves; eight dairy calves, with a mean (SD) weight of 56 (7) kg and ranging in age from 4–8 weeks. During the experiments, the calves were kept in individual pens. They were not fasted prior to the experiments.

Cows; eight dairy cows with a mean (SD) weight of 590 (42) kg and a daily milk yield of 25.4 (5.4) kg. The animals were kept in a conventional tie stall barn throughout the study period. Although not fasted prior to the experiments, the cows were not permitted to eat grain on the days on which the experiments took place.

Sheep; eight ewes, with a mean (SD) weight of 81 (3) kg were included in the study. The sheep had been sheared approximately 1 week prior to the start of the study. They were not fasted prior to the experiments and were kept in individual pens adjacent to other sheep for the duration of the experiments.

All the animals were included in a cross-over pharmacokinetic study on medetomidine and atipamezole, the results of which are reported elsewhere (Ranheim *et al.*, 1998, 1999, 2000). The study was approved by the Norwegian Animal Research Authority.

Drug treatments and study design

The design of the study was a simple crossover trial in which each animal received the following treatments: medetomidine, 40 μg/kg i.v. (Domitor® 1 mg/mL; Orion Corporation Animal Health, Turku, Finland), followed 60 min later by atipamezole, 200 μg/kg i.v. (Antisedan® 5 mg/mL; Orion Corporation), or an equal volume of saline, after 60 min. The treatment order in each animal was randomized, and the wash out period between experiments was 1 week for the calves and sheep, and 2 weeks for the cows.

Blood sampling

Medetomidine and atipamezole or saline was injected by venipuncture into the right jugular vein. In sheep and cows, catheters (Secalon® T, gauge 16) with extension tubes (Connecta®, 25 cm) were placed in the left jugular vein and flushed with heparinized saline. The extension tube was taped to the dorsal aspect of the neck. The catheters and extension tubes were used to sample blood (2×5 mL) into EDTA tubes prior to injection of medetomidine and at 5, 25, 45, 65, 85, 95, 105, 120, 180, 240, 300, 360, 420 and 480 min after injection. In the calves, the same sampling schedule was used and samples of blood (5 mL) were collected into EDTA tubes from the right jugular vein using Venoject® syringes. The blood samples were immediately cooled on ice, and plasma was subsequently separated by centrifugation at $1500 \times \boldsymbol{g}$ for 10 min, and stored at − 70 °C until assayed.

Chemical analyses

Catecholamines in plasma from cows and sheep were determined using high-performance liquid chromatography (HPLC) with electrochemical detection (Kågedal & Goldstein, 1988; Musso *et al.*, 1990). Plasma samples from the calves were not assayed for catecholamines. Plasma glucose was measured using an enzymatic method that involves the conversion of glucose to 6-phosphogluconate and NADH. The amount of NADH produced is directly proportional to the concentration of glucose present in the sample and is measured by its absorbance at 340 nm (Technicon Axon® Systems, Tarrytown, NY, USA). Cortisol concentrations in sheep plasma were analysed using a commercial cortisol [^{125}I] radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA, USA), in which the ^{125}I-labelled cortisol competes with the sample cortisol for antibody immobilized to the wall of a polypropylene tube. After decanting the supernatant, the radioactivity is measured in a gamma counter. Cortisol concentrations in cow and calf plasma was analysed using a competitive immunoassay technique (Amerlite Cortisol Assay; Ortho-Clinical Diagnostics, Amersham, UK). Cortisol in plasma competes with horseradish peroxidase (HRP)-labelled cortisol for a limited number of binding sites on a sheep anti-cortisol antibody presented in the liquid phase. The HRP activity of the bound conjugate is measured by an enhanced luminescence reaction.

Statistical analyses

In order to decide whether a mean difference from baseline to 25 min after medetomidine injection within an animal group was statistically significant, a two-sided Wilcoxon signed-rank test was applied. When comparing medetomidine followed by atipamezole and medetomidine followed by saline within an animal group, an ANOVA repeated measures model was used (SAS® 6.11 statistical software; SAS Institute Inc., Cary, NC, USA) with a Bonferroni correction. Differences were considered significant when $P < 0.05$.

RESULTS

In both cattle and sheep the injection of medetomidine induced a marked and statistically significant hyperglycaemia. In calves and cows, the maximum values were reached 120 and 105 min after the injection of medetomidine, respectively (Fig. 1a, b). All reported values are given as mean (SD). The maximum values of plasma glucose were similar in calves and cows, being 10.4 (3.0) and 11.1 (3.7), respectively. In sheep, the increment was smaller (Fig. 1c). After the initial rise, the plasma glucose stabilized until 105 min after the injection of medetomidine, but after this time there was a secondary rise, plasma glucose increasing further, reaching a maximum of 9.6 (2.0) mmol/L at 240 min. Injection of atipamezole clearly antagonized the hyperglycaemic effect of medetomidine in calves and cows. In sheep, the injection of

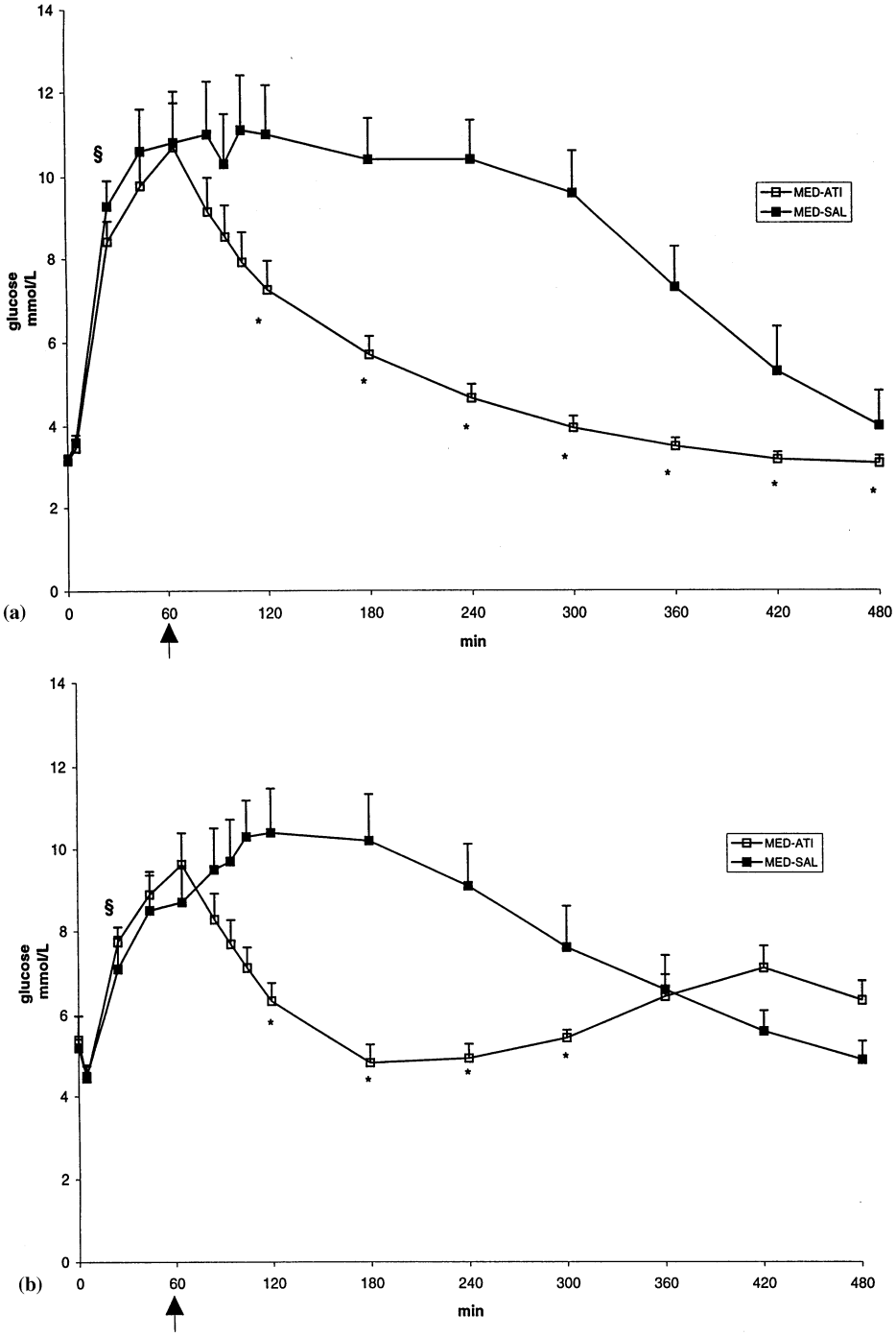


Fig. 1.

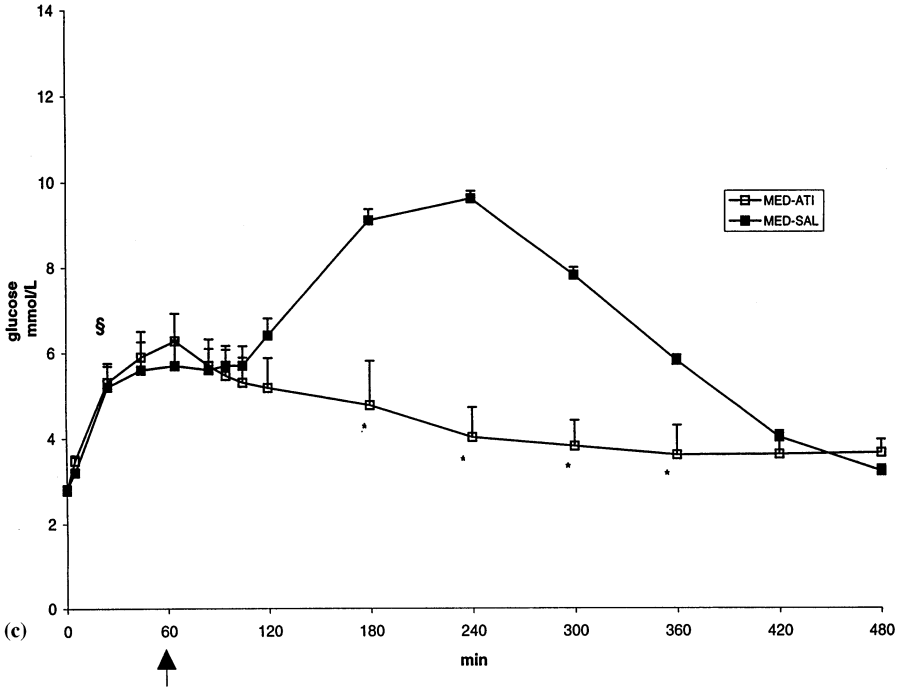


Fig. 1. Mean + SEM plasma glucose of eight dairy cows (a), calves (b) and sheep (c). Medetomidine (40 μg/kg i.v.) was given at time 0, at 60 min it was followed by atipamezole (200 μg/kg i.v.) [MED-ATI] or saline [MED-SAL] (arrow). § MED-ATI and MED-SAL at 25 min are significantly different from time 0. * MED-SAL is significantly different from MED-ATI at same time point.

the antagonist did not reduce the hyperglycaemia to the same extent, but prevented the second rise in the plasma glucose seen after administration of medetomidine followed by saline.

The effect of medetomidine and atipamezole on cortisol levels seemed to be somewhat different in calves, cows and sheep. In calves, the plasma cortisol level increased slightly after medetomidine injection, but this rise was not statistically significant ($P = 0.07$) (Fig. 2a). The cortisol level in the cows increased almost 4-fold from baseline, to a maximum at 45 min (Fig. 2b). In the sheep, the increments were 6–8-fold, the highest value being observed 25 min after medetomidine injection (Fig. 2c). The injection of atipamezole had no statistically significant effect on the cortisol level in cows or calves. In sheep, however, there was an increase that was statistically significant. The cortisol levels in all animals at 120 min after medetomidine injection were lower when the animals were injected with atipamezole compared with saline injection, but this was not significant in calves.

In both cows and sheep, the injection of medetomidine caused a significant decrease in the concentration of noradrenaline in plasma. When atipamezole was injected i.v. into cows and sheep, plasma noradrenaline increased to 1432 and 2267 pg/mL respectively, representing a more than 4-fold increase from baseline (Fig. 3a, b). An interfering peak in the chromatograms unfortunately, hampered the electrochemical detection of adrenaline for many of the samples analysed. In three sheep, the adrenaline concentration ranged from 8–15 pg/mL at 45 min after medetomidine injection, increasing to (range) 283–429 pg/mL 5 min after atipamezole was injected. In cows, the effect on adrenaline concentration was smaller, an increase from a mean of 13–186 pg/mL being observed after the injection of atipamezole in five animals.

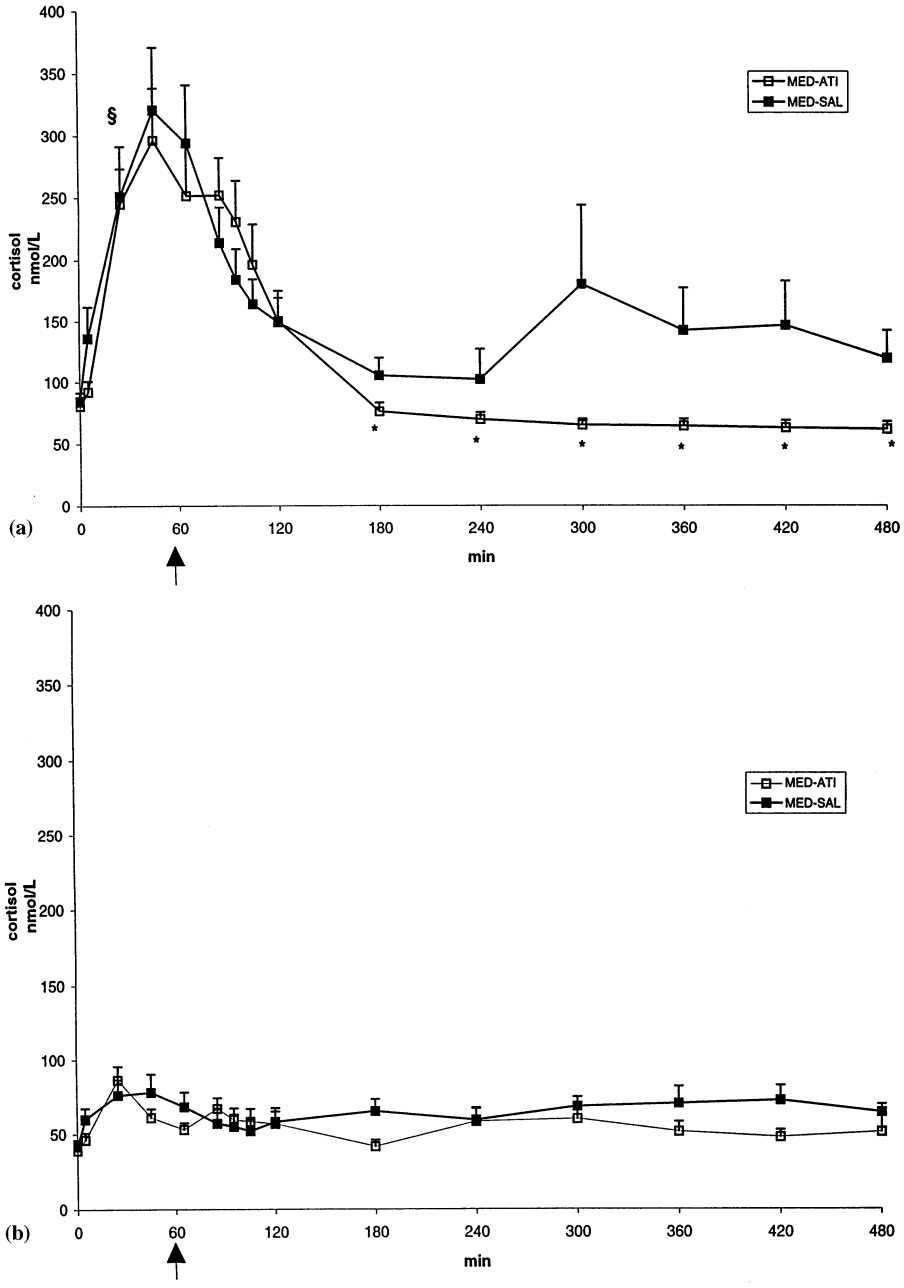
DISCUSSION

It has been shown *in vitro* that xylazine inhibits the release of insulin by activation of α_2-receptors on pancreatic β-cells (Abdel El Motal & Sharp, 1985). The present results confirm earlier reports on the hyperglycaemic effects of α_2-agonists. Previous studies in ruminants report significant hypoinsulinaemia and hyperglycaemia in sheep (Brockman, 1981), calves (Kasuya *et al.*, 1996a,b) and cows (Hsu & Hummel, 1981) after administration of xylazine. The stepwise increase in glucose concentration observed in sheep in this study after the administration of an α_2-agonist has, to our knowledge, not previously been described. Brockman (1981) injected sheep with 0.16 mg xylazine/kg i.v. and reported a gradual increase in glucose concentration from 15 min post injection to a maximum at 30 min. In that study, xylazine induced hypoinsulinaemia and hyperglucagonaemia. The increase in glucagon was of shorter duration than the decrease in insulin concentration. If medetomidine induces the same alterations in pancreatic hormones in sheep as xylazine, the second increase in glucose concentration could possibly be explained by a normalized glucagon level and depressed insulin concentration 2 h after medetomidine injection. In cattle, the hyperglycaemia induced by xylazine (Eichner *et al.*, 1979; Hsu & Hummel, 1981) seems to be of longer duration than the hyperglycaemia induced by medetomidine in this study. This might be attributable to the α_1-agonist activity of xylazine in the liver, inducing glucose production as the α_1-antagonist prazocin reduced the xylazine induced hyperglycaemia in cattle (Hsu & Hummel, 1981).

Whereas atipamezole had a marked effect on the hyperglycaemia induced by medetomidine in cows and calves, this effect was not so well defined in sheep. Most likely, atipamezole

reverses the hypoinsulinaemia induced by medetomidine, although somewhat conflicting results on the effect of α_2-antagonists on insulin secretion have been reported. Yohimbine reversed the hypoinsulinaemia in cattle and dogs (Hsu & Hummel, 1981; Hsu, 1988), whereas Kasuya *et al.* (1996b) reported that atipamezole failed to reverse the hypoinsulinaemia induced by xylazine in calves.

Basal cortisol values in cows and calves range from 5–30 nmol/L (Nicolic *et al.*, 1998), and in sheep it is reported to be approximately 20 nmol/L (Niezgoda *et al.*, 1993). In this study the pretreatment cortisol values in cattle and sheep were slightly higher, which may indicate that the sampling procedure was somewhat stressful for the animals. The significant increase in cortisol concentration in cows and sheep found in this study may be explained by the high dose of medetomidine used. Kallio *et al.* (1988) reported a dose-dependent decrease in cortisol concentrations in humans after injection of medetomidine and Benson *et al.* (2000) reported that medetomidine decreased surgically induced increments in cortisol concentrations in dogs. Also, Raekallio *et al.* (1992) described a decrease in cortisol concentration, while Carroll *et al.* (1997) did not observe any effects of detomidine on cortisol levels in six horses. Clonidine has a well-documented inhibitory effect on ACTH secretion (Ganong, 1980). Low doses of xylazine have also been shown to inhibit the stress-induced increase in cortisol concentration in cattle and goats (Brearley *et al.*, 1990; Sanhouri *et al.*,



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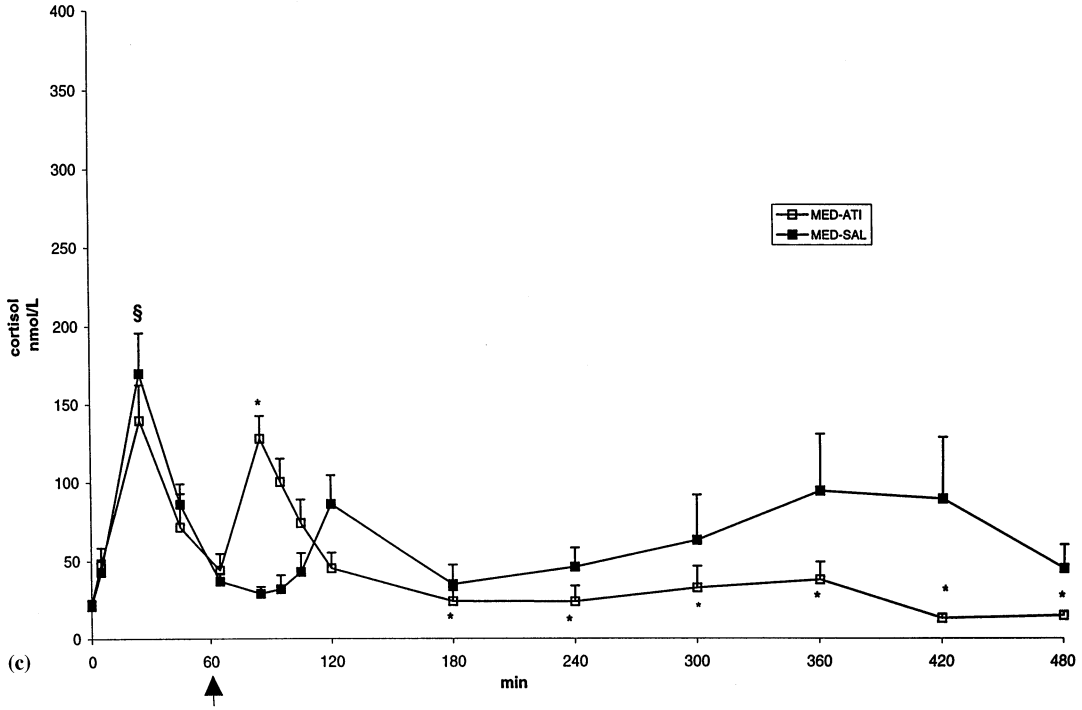

Fig. 2. Mean + SEM plasma cortisol of eight dairy cows (a), calves (b) and sheep (c). Medetomidine (40 μg/kg i.v.) was given at time 0, at 60 min it was followed by atipamezole (200 μg/kg i.v.) [MED-ATI] or saline [MED-SAL] (arrow). § MED-ATI and MED-SAL at 25 min are significantly different from time 0. * MED-SAL is significantly different from MED-ATI at same time point.

1992). Imidazoline compounds, such as medetomidine, are also reported to have an inhibitory effect on steroidogenesis (Maze *et al.*, 1991).

Increases in ACTH and subsequently cortisol, occur rapidly when physiological strain such as hypoxia, hypotension and hypoglycaemia are induced by various stressors (Kemppainen & Behrend, 1997). Alpha$_2$-adrenergic agonists cause bradycardia, an initial hypertenson followed by hypotension (Bryant *et al.*, 1996) and in ruminants, hypoxaemia is also a well-documented side effect (Celly *et al.*, 1997). A significant depression of the cardiovascular system most likely occurred with the high dose of medetomidine applied. α_2-Agonists induce hypoxaemia in cattle and sheep (De Moor & Desmet, 1971; Bryant *et al.*, 1996; Celly *et al.*, 1997). Hypotension combined with hypoxaemia could possibly override any depressant effect of the α_2-agonist on ACTH secretion (Kallio *et al.*, 1988; Raekallio *et al.*, 1992) and might explain the increase in cortisol observed in ruminants. An increase in cortisol was also observed in reindeer injected with medetomidine (Arnemo & Ranheim, 1999). The results collected in this study indicate the existence of an age or species difference in the endocrine effects of α_2-agonists. Whether the drug acts at one or several levels in the HPA axis or by an indirect mechanism could not be determined.

When horses sedated with detomidine were injected with the α_2-antagonist tolazoline, the cortisol concentration also increased (Carroll *et al.*, 1997). An increase in the cortisol levels after the injection of atipamezole was therefore expected, but this immediate effect was observed only in sheep. Atipamezole apparently reduced the cortisol level from 2 h after its injection in sheep and cows, indicating a prolonged action of the antagonist on cortisol secretion. We have speculated that the slow return of plasma glucose to baseline in sheep injected with atipamezole, maybe attributable to the increase in cortisol associated with injection of the antagonist. Cortisol increases plasma glucose levels by inhibiting glucose utilization in the tissues and increasing glycogenolysis in the liver. The large increase in catecholamines could also possibly enhance this effect (Breazile, 1987; Dickson, 1993).

Plasma noradrenaline does not seem to originate from within the CNS but largely represents the transmittor released by sympathetic nerve terminals in the sympathetic nervous system, of which a small fraction spills over into the venous drainage (Esler *et al.*, 1984). A small amount of noradrenaline is also derived from the adrenal medulla. In cows and sheep, the percentage of the total amount of catecholamines released from the adrenal medulla as noradrenaline has been reported to be 29 and 33%, respectively (Dickson, 1993). A reduction in plasma catecholamines after the injection of medetomidine was anticipated, as the drug reduces the release of noradrenaline in the sympathetic nervous system. This has also previously been reported in horses injected with detomidine (Raekallio *et al.*, 1991, 1992) and in humans administrered medetomidine (Scheinin *et al.*, 1989) or dexmedetomidine (Scheinin *et al.*, 1998). Clinically, calves, cows and sheep in particular, showed some excitement after the injection of atipamezole (unpublished results; Ranheim *et al.*, 1999, 2000). The large increase in plasma catecholamines was comparable to the increase in noradrenaline and adrenaline observed in humans

after infusion of atipamezole (Scheinin *et al.*, 1998), reflecting the large increase in sympathetic activity induced by the i.v. injection of the antagonist. The larger increase in catecholamine levels observed in sheep might, therefore, be associated with the higher level of excitement observed clinically in this species. In contrast, Carroll *et al.* (1997) reported decreasing concentrations of adrenaline over time and no change in noradrenaline, irrespective of the injection of the α_2-antagonist tolazoline to six horses sedated with detomidine. These differences may be attributable to the different α_2/α_1-selectivity of the drugs, dose effects, or to species differences.

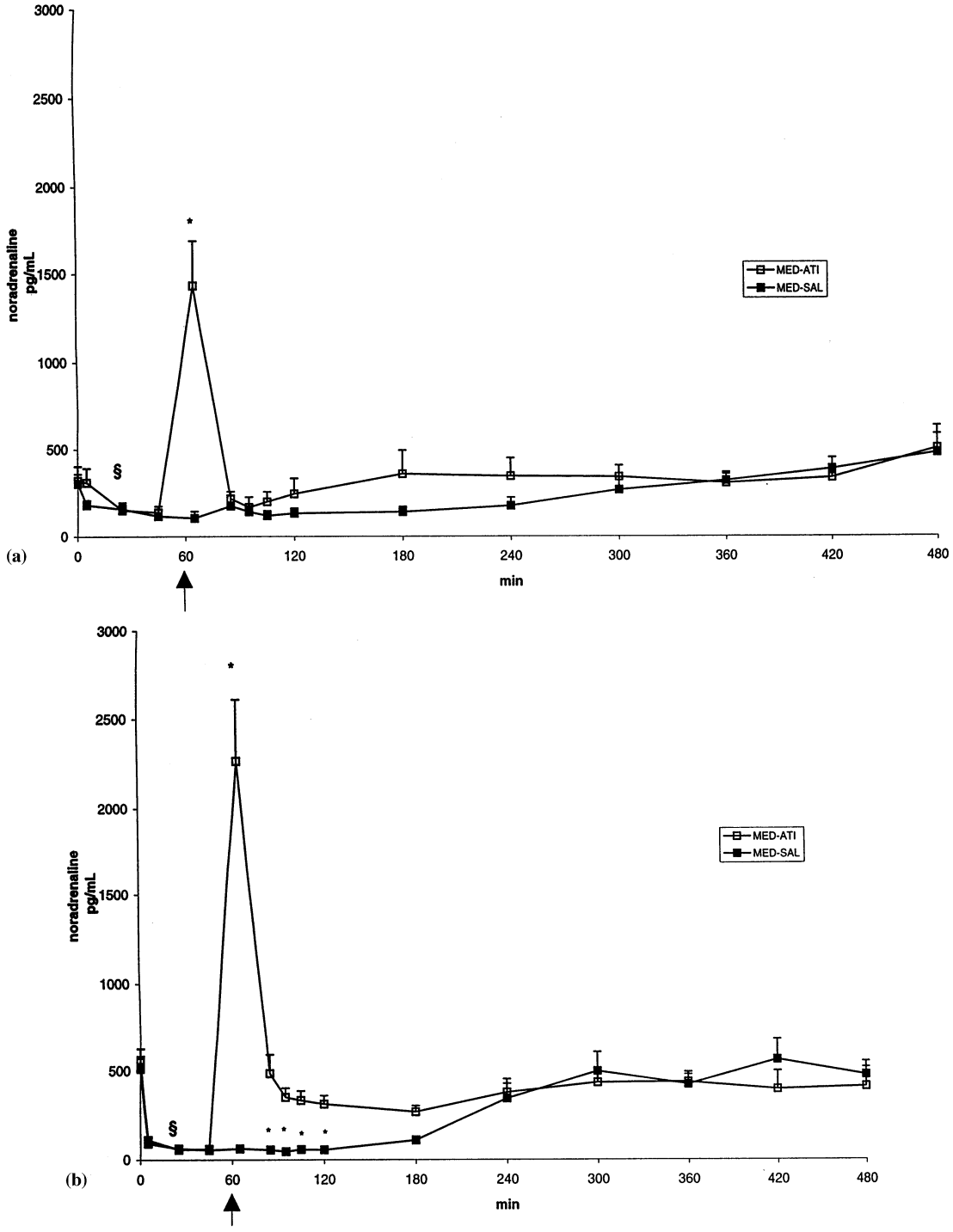

Fig. 3. Mean + SEM plasma noradrenaline of eight dairy cows (a) and sheep (b). Medetomidine (40 μg/kg i.v.) was give at time 0, at 60 min it was followed by atipamezole (200 μg/kg i.v.) [MED-ATI] or saline [MED-SAL] (arrow). § MED-ATI and MED-SAL at 25 min are significantly different from time 0. * MED-SAL is significantly different from MED-ATI at same time point.

In conclusion, the effects of medetomidine and atipamezole on plasma cortisol seem to be different in cattle and sheep compared to other species. As α_2-agents are regularly used in ruminants, further investigations into the mechanisms involved in the endocrine and metabolic effects of these drugs should be conducted.

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