The α_2-adrenoceptor agonists xylazine and guanfacine exert different central nervous system, but comparable peripheral effects in calves

G. SCHOLTYSIK*
F. REGLI*
R. M. BRUCKMAIER† &
J. W. BLUM†

Institute of Veterinary Pharmacology and †Division of Nutrition Pathology, Institute of Animal Breeding, University of Berne, Switzerland

Scholtysik, G., Regli, F., Bruckmaier, R. M., Blum, J. W. The α_2-adrenoceptor agonists xylazine and guanfacine exert different central nervous system, but comparable peripheral effects in calves. *J. vet. Pharmacol. Therap.* **21,** 477–484.

Acute pharmacodynamic effects of the α_2-adrenoceptor agonists, xylazine and guanfacine, were investigated in nine healthy calves in an open crossover trial. Xylazine (100 μg/kg body weight intravenously (i.v.)) and guanfacine (20 μg/kg body weight i.v.) were equi-effective in lowering heart rate by 25–30% at 5 min. Under these conditions, xylazine induced strong sedation and increased plasma growth hormone levels, indicating central nervous system mediated actions, whereas guanfacine was not sedative and did not induce release of growth hormone. Oxygen consumption was decreased by both drugs, but respiratory exchange ratio decreased only in response to xylazine. However, in response to both drugs, plasma levels of noradrenaline, adrenaline, insulin and non esterified fatty acids decreased similarly and glucose increased comparably. These results demonstrate marked differences in the central nervous system-mediated effects of the two α_2-adrenoceptor agonists, whereas peripheral actions are similar.

(Paper received 10 February 1998; accepted for publication 2 July 1998)

Prof. Dr Günter Scholtysik, University of Berne, Institute of Veterinary Pharmacology, Länggass-Strasse 124, CH-3012 Bern, Switzerland.

INTRODUCTION

Alpha$_2$-adrenoceptor agonists, such as xylazine, medetomidine or romifidine are widely used as sedatives and analgesics in clinical veterinary medicine. They belong to a large group of sympathoinhibitory drugs, of which other members, such as clonidine or guanfacine, are used as antihypertensives in humans (Scholtysik, 1992). Alpha$_2$-adrenoceptor ligands as well as α_2-adrenoceptors are heterogeneous. Alpha$_2$-adrenoceptor subtypes have been identified and named α_{2A}, α_{2B} and α_{2C} (Harrison *et al.*, 1991; Bylund *et al.*, 1994; for review see MacDonald *et al.*, 1997). A fourth subtype, α_{2D}, is considered to be the bovine and rodent homologue of the α_{2A} adrenoceptor found in humans, pigs and rabbits (Bylund *et al.*, 1995). Their tissue distribution and functional role was extensively investigated (Ruffolo *et al.*, 1993), but basic questions still remain open. Thus, α_2-adrenoceptor agonists elicit different affinities to α_2-adrenoceptor subtypes (Hieble & Ruffolo, 1996). For example, guanfacine and xylazine have preferential affinities to α_{2A}-adrenoceptors (MacLennan *et al.*, 1997) and α_{2D}-adrenoceptors (Smith *et al.*, 1995), respectively. It is therefore not surprising that different α_2-adrenoceptor agonists exert variable pharmacological effects, for example, with respect to sedation. It is known that xylazine is sedative not only in animals but also in humans (Kroneberg *et al.*, 1967), in contrast to guanfacine which is hardly sedative in humans (Nami *et al.*, 1983) and laboratory animals (Kleinlogel *et al.*, 1975). The marginal sedative effects of guanfacine encouraged Hunter (1992) and later Miaron *et al.* (1994) to investigate its metabolic effects in steers in order to utilise sympathoinhibition for energy saving. It has been found that guanfacine decreases energy expenditure without sedation (Hunter, 1992). Based on that we have compared acute sedative, cardiorespiratory, metabolic and endocrine effects of both xylazine and guanfacine.

MATERIALS AND METHODS

Animals and experimental protocols

The experiments were performed with nine healthy, 2.5–3.5 months old male Simmentaler × Red Holstein calves. The animals were raised in a loose housing system and fed twice daily a standard milk replacer used for veal calves (purchased from Provimi SA., Cossonay-Gare, Switzerland) according to manufacturers recommendations. Body weight (BW) ranged from 96 to 129 kg (mean: 114 ± 4 kg) at the start of experiments. Experimental protocols were approved by the cantonal and federal committees for permission of animal experiments.

The evening before drug applications, the animals were moved into single boxes and indwelling catheters were inserted into the left jugular vein. Drugs were always administered in the morning after a period of 16–18 h without feed. The study

was designed in a manner that all treatments (xylazine, *group X*; guanfacine, *group G*; or sodium chloride, *group C*) were applied to all calves in a random manner (open crossover) and that each animal served as its own control. The interval between experiments for recovery from treatments was at least 7 days. Drugs (100 μg xylazine/kg BW; 20 μg guanfacine/kg BW) were injected intravenously (i.v.) over 60 sec *via* the implanted catheter, followed by washing with 3 mL sodium chloride (9 g/L). Sodium chloride (10 mL) was injected i.v. in controls.

Blood samples (20 mL) were collected 20 min before and after the administration of xylazine, guanfacine or sodium chloride. Blood samples were taken into tubes containing sodium-EDTA (2.25 mg/mL blood) and heparin, respectively, and centrifuged at $3000\times$ ***g*** for 10 min, the plasma harvested and frozen in aliquots of 2 mL at -80°C for later analysis.

Heart rate was measured immediately before taking blood samples. The goal was to have equal bradycardic effects of both drugs. Based on preliminary dose-finding studies in three animals (involving 5–30 μg/kg BW guanfacine i.v. and 100 μg/kg BW xylazine i.v.) 100 μg xylazine and 20 μg guanfacine/kg BW, both lowered heart rate by 25–30% at 5 min after administration. These doses were therefore chosen to study guanfacine and xylazine effects.

One month after the first experiment all animals were additionally i.v. injected with xylazine, guanfacine or NaCl in the same amounts as described above to study respiratory effects.

Sedation was assessed before and at 5, 10, 15, 30, 45 and 60 min after drug administration. As the degree of sedation is not measurable objectively, we have considered the body position (standing, lying), eye lid positions (open, closed) and movements, head position (upright, subside), ear movements and reaction to environmental events (moderate noise), in order to assess the degree of sedation on an arbitrary 1–4-point rating scale where 1 was slight, 2 was medium, 3 was moderate and 4 was strong as the most evident sedation with lying down.

Drugs

Guanfacine [*N*-amidino-2-(2,6-dichlorophenyl)acetamide hydrochloride] was a generous gift from Sandoz AG, Basle, Switzerland

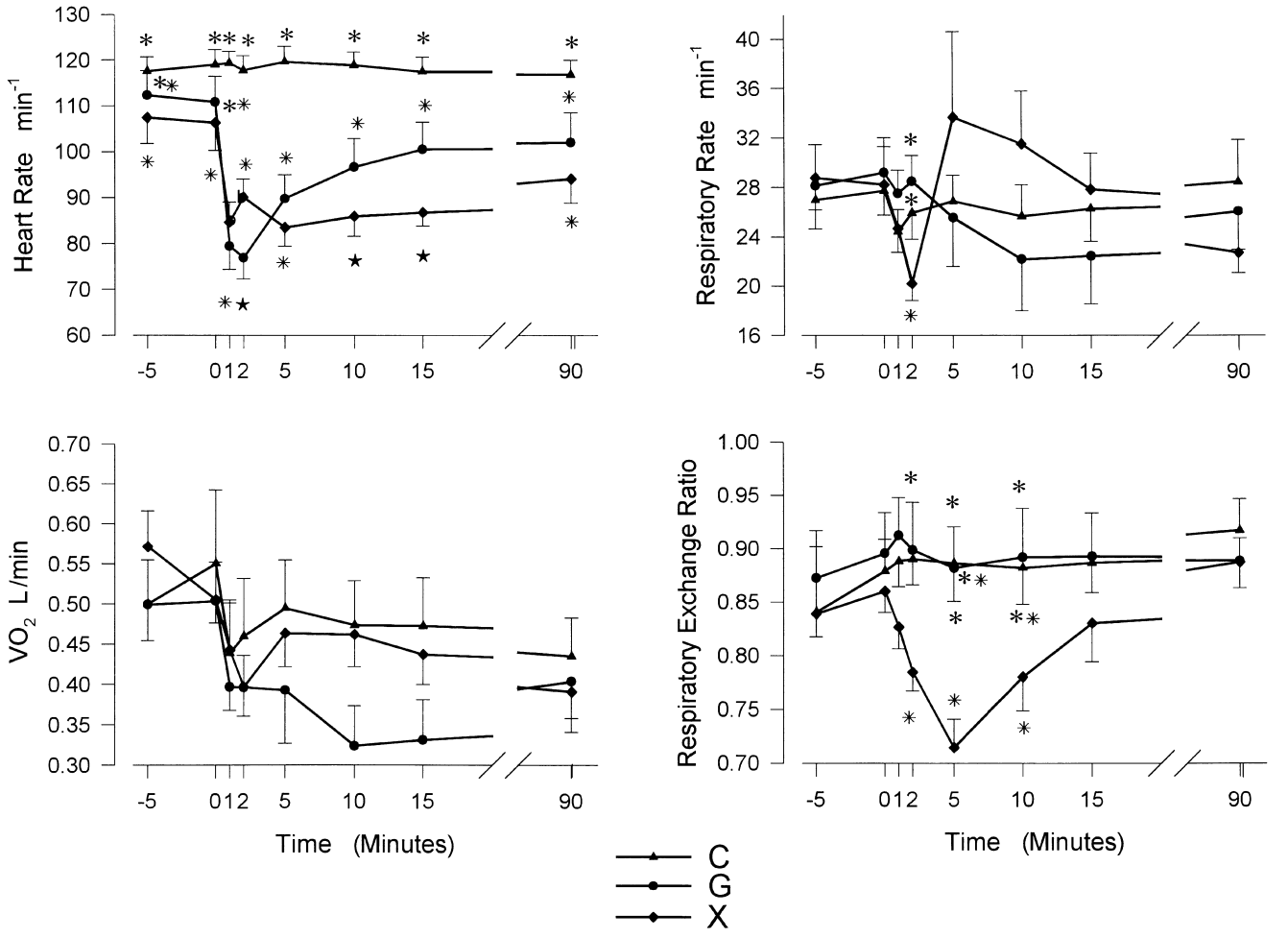

Fig. 1. Heart rate, respiratory rate, respiratory exchange ratio and oxygen consumption (VO_2 in litres per minute) in calves treated with xylazine (group X; 100 μg/kg i.v., ◆) or guanfacine (group G; 20 μg/kg i.v., ●) or sodium chloride solution (group C; 10 mL i.v., ▲) at time zero. Each point represents the mean ± SEM value of 9 animals. Means without common asterisks (✻ ✳ ★) are significantly different ($P < 0.05$) between groups on different time points.

and was dissolved in NaCl (0.25 mg/mL). Xylazine [2-(2,6-xylidin)-5,6-dihydro-4H-1,3-thiazine hydrochloride], 1 mg/mL as Rompun® was purchased from Bayer AG, Leverkusen, Germany.

Laboratory methods

Adrenaline (A) and noradrenaline (NA) were determined in blood plasma after extraction (Ganhao *et al.*, 1991) by high performance liquid chromatography (HPLC) and electrochemical detection (Gerlo & Malfait, 1985; Horvai & Pungor, 1989). Plasma growth hormone (GH) (Zimmerli & Blum, 1990), insulin (Blum & Flückiger, 1988), non esterified fatty acids (NEFA) (Bruckmaier & Blum, 1992) and glucose concentrations (Hostettler-Allen *et al.*, 1994) as well as oxygen consumption (VO_2), carbon dioxide production, respiratory exchange ratio (RER) (Blum & Flückiger, 1988; Zimmerli & Blum, 1990; Bruckmaier & Blum, 1992) and respiratory rate were measured as previously described.

Statistical analyses

Data are presented as means and standard errors (SEM). The values presented for cardio-respiratory parameters at 5, 10, 15 and 90 min are individual means of 2-min periods (3–5, 8–10, 13–15 and 88–90 min, respectively). For statistical evaluation, repeated measurement analysis of variance was calculated using the GLM procedure of SAS (1989) Version 6.11 1995. The model used was Y (repeated time) = animal_i + treatment_j + $\text{residual error}_{ijk}$. Linear contrasts were calculated between time '0' and all other time points. If 'time–treatment' interactions were significant ($P < 0.05$), treatment differences within a time point were post hoc localized using the Least Significant Difference (LSD) test (SAS, 1989). The results of the LSD test are indicated on Figs 1–5.

RESULTS

Heart rate, respiratory rate, oxygen consumption and respiratory exchange ratio (Fig. 1)

Heart rate decreased ($P < 0.05$) within 1–2 min after xylazine and guanfacine injections, but remained unchanged in group C. At 2 min after injections, heart rate was lower ($P < 0.05$) in group G than in group X. After 5 min heart rate increased in group G, whereas it remained low in group X ($P < 0.05$). At 10 and 15 min heart rate was higher ($P < 0.05$) in group G than in group X. At 90 min heart rate was no longer different in groups X and G, but was still lower ($P < 0.05$) in both groups than in group C.

Respiratory rate in group X slightly decreased ($P < 0.05$) within 2 min and then nonsignificantly increased above pretreatment values at 5 min after injection, but did not change in groups G and C. Values in groups X and G were not different from those in group C.

Oxygen consumption (VO_2) decreased ($P < 0.05$) within 1 min after drug injections in all treatments and remained lower ($P < 0.05$) than pretreatment values throughout the experiments. There were no significant group differences.

Respiratory exchange ratio in group X decreased ($P < 0.05$) up to 5 min after the injection and then returned to pretreatment values at 15 min, whereas it did not change in groups G and C.

Sedation (Fig. 2)

Sedation increased ($P < 0.05$) in groups X and G and was maximal between 5 and 15 min after injections, whereas behaviour remained unaffected in group C. Sedation up to 45 min was more pronounced ($P < 0.05$) in group X than in group G, but was still greater ($P < 0.05$) in group G than in group C at 90 min

Plasma catecholamines (Fig. 3)

Adrenaline was significantly ($P < 0.05$) decreased in response to xylazine by more than 50% within 30 min after treatment and to guanfacine by 40% within 15 min after treatment. Levels of adrenaline remained decreased during 180 min after treatment with both drugs and then returned slowly to control levels within 24 h after treatment. Plasma levels of noradrenaline were decreased after xylazine and guanfacine administration but this was not statistically significant when compared with the sodium chloride treated control group.

Plasma insulin and growth hormone (Fig. 4)

Insulin concentrations, after a decrease within 30 min, increased in groups G and X to a peak at 150 min after injections and then

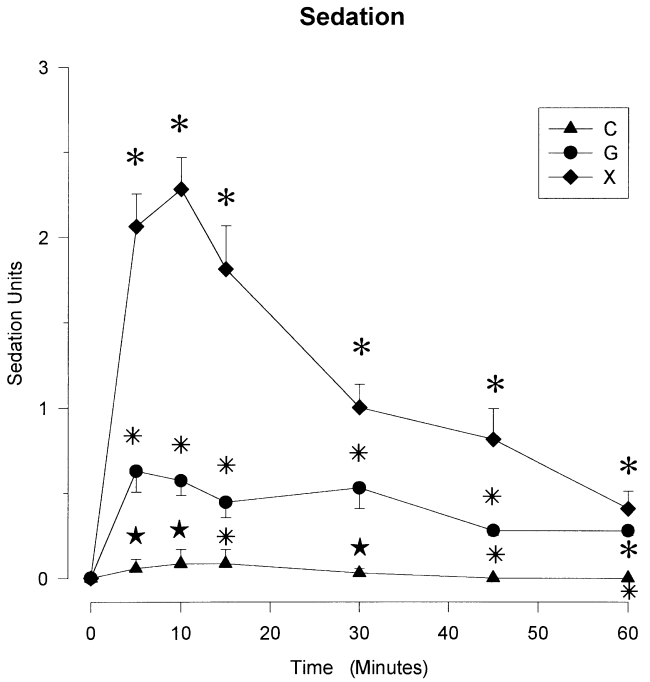

Fig. 2. Sedation (arbitrary rating units) in calves treated with xylazine (◆) or guanfacine (●) or sodium chloride solution (▲) at time zero. Each point represents the mean ± SEM value of 9 animals. For details: see legend to Fig. 1.

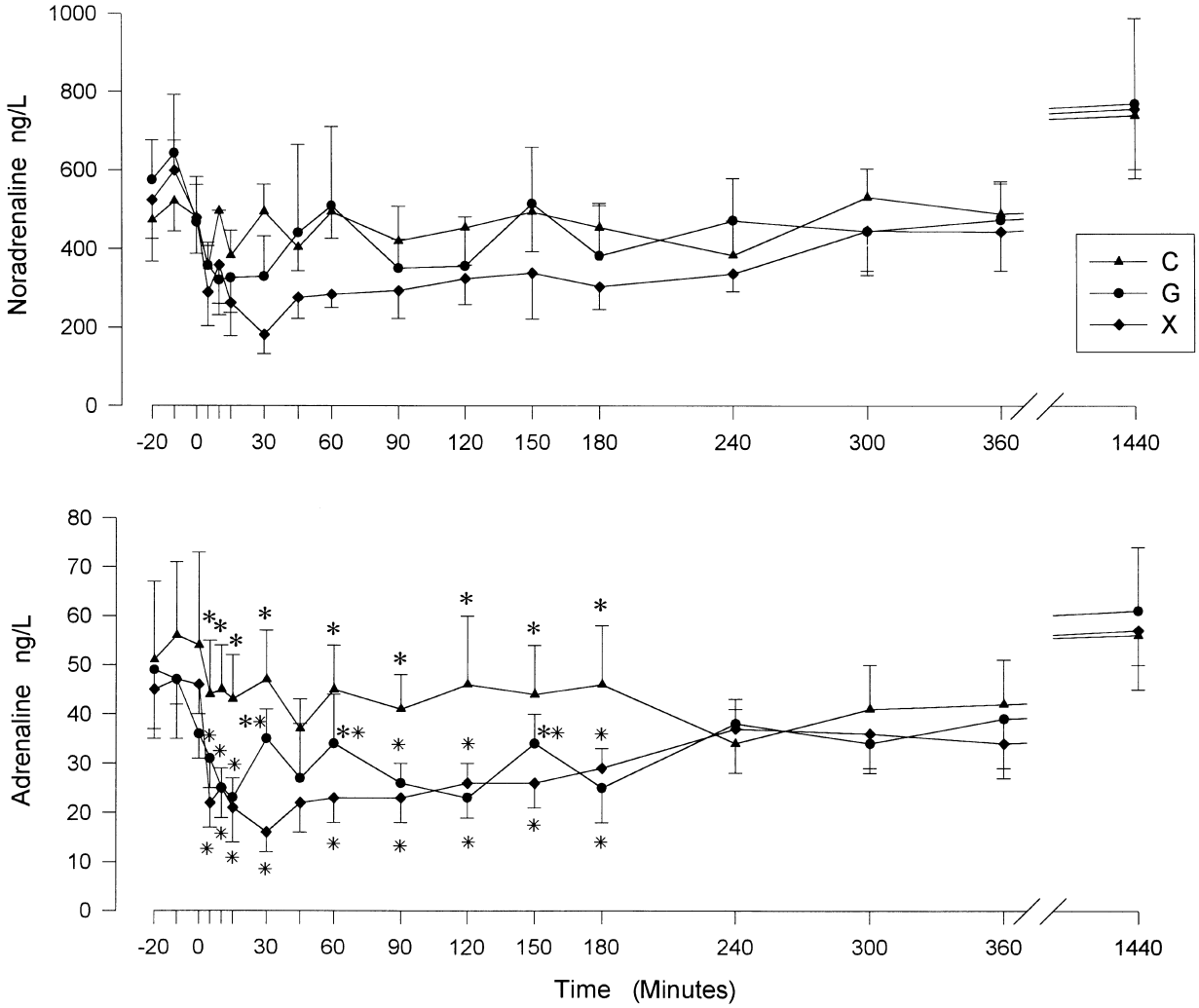

Fig. 3. Plasma levels of adrenaline and noradrenaline in calves treated with xylazine (◆) or guanfacine (●) or sodium chloride solution (▲) at time zero. For details see legend to Fig. 1.

decreased again. Due to large variations between animals, changes in groups X and G were not statistically significant. Insulin levels in group C remained stable throughout the experiment.

Growth hormone levels increased ($P < 0.05$) 5.5-fold in response to xylazine injections to a peak after 30 min and then returned to pretreatment values within 90 min. In groups G and C, concentrations of GH remained on baseline levels throughout the experiment.

Plasma glucose and non esterified fatty acids (Fig. 5)

Basal glucose concentrations were lower ($P < 0.05$) in group C than in groups G and X. Although levels did not change in group C, concentrations in groups G and X increased transiently within 5 min up to 45 min after injections, then decreased and at 240 min reached baseline levels. The decrease was faster in group G than in group X and at 120 min concentrations were lower ($P < 0.05$) in group G than in group X.

Concentrations of NEFA in groups X and G decreased ($P < 0.05$) from pretreatment levels by about 50%, remained low up to 180 min and then returned to levels of group C within 6 h after injections. In group C, NEFA transiently increased immediately after NaCl injections and from 150 min NEFA increased slightly up to 6 h after treatments.

DISCUSSION

The selection of guanfacine for the present investigation was based on previous observations demonstrating decreases in metabolic rate in steers (Hunter, 1992; Hunter *et al.*, 1993; Miaron *et al.*, 1994; Gazzola *et al.*, 1995) and rats (Gazzola, 1993, 1995). Of available α_2-agonists the antihypertensive guanfacine was the only one lacking sedation. Xylazine was selected for our comparative study as the classical representative of sedative α_2-agonists.

The doses we have applied were chosen to be about equi-effective with respect to heart rate lowering. This was achieved in preliminary dose-finding experiments with 100 µg xylazine/kg BW i.v. and 20 µg guanfacine/kg BW i.v. at 5 min after the

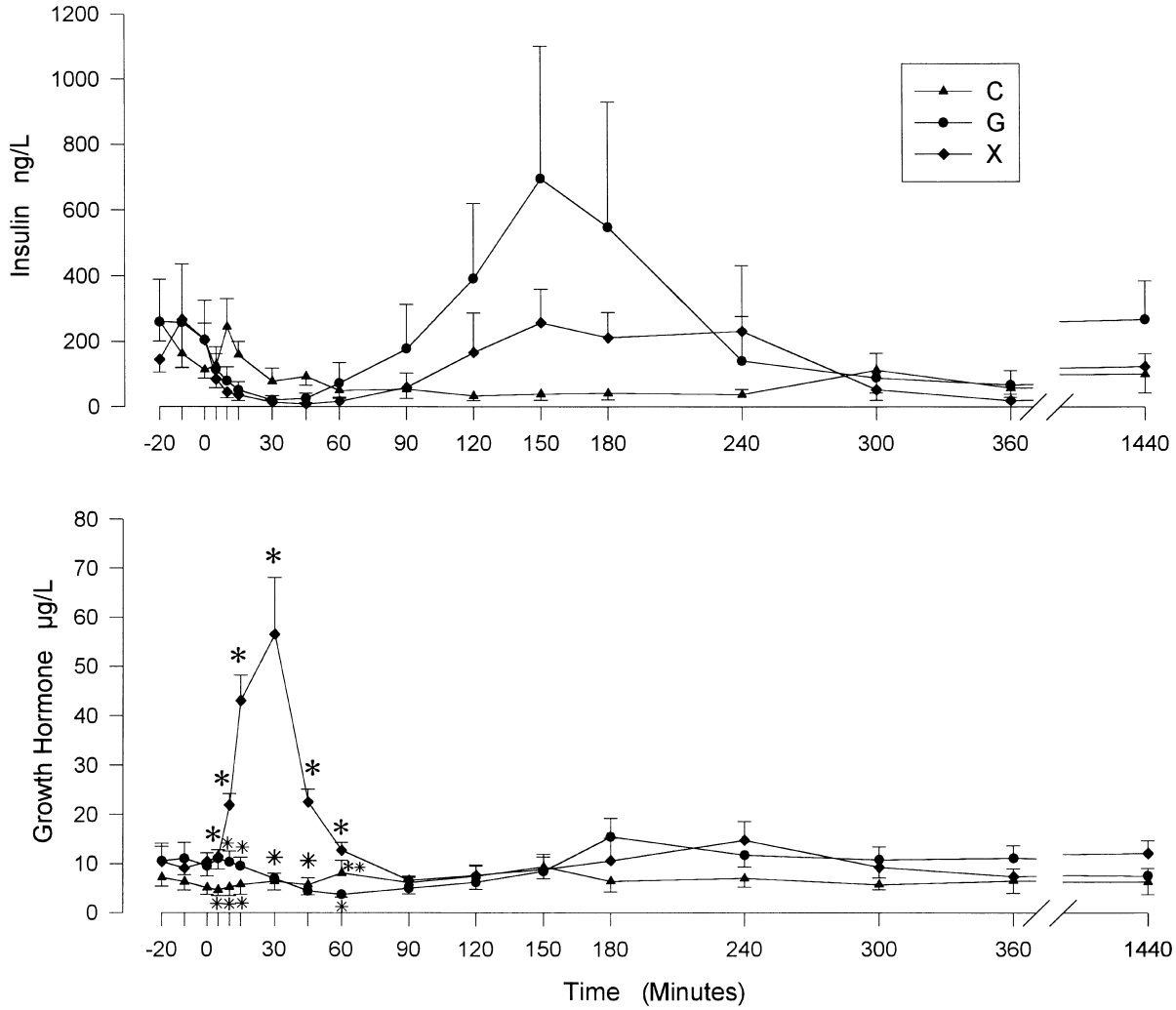

Fig. 4. Plasma levels of growth hormone and insulin in calves treated with xylazine (◆) or guanfacine (●) or sodium chloride solution (▲) at time zero. For details see legend to Fig. 1.

injection. In the main experiment, however, the heart rate was lower from 10 min onward with xylazine. These doses were in the order of magnitude used by other authors for xylazine (Roming, 1984; Kümper, 1989; Kyles *et al.*, 1993) and for guanfacine (Hunter, 1992; Gazzola *et al.*, 1993; Miaron *et al.*, 1994; Gazzola *et al.*, 1995).

The main message of the present investigation was the observation that the two α_2-adrenoceptor agonists, xylazine and guanfacine, differ qualitatively in their acute pharmacological effects in calves. Thus, xylazine induced massive sedation and enhanced plasma GH levels, whereas guanfacine lacked such effects. On the other hand, xylazine and guanfacine had comparable effects on plasma glucose, NEFA, NA, A, and insulin plasma concentrations, as well as on respiratory rate, VO_2 and RER.

We consider sedation and GH secretion as CNS-mediated effects. Sedation observed after xylazine administration confirmed widespread experience (Roming, 1984; Kümper, 1989). In contrast to xylazine, guanfacine hardly induced sedation, thus confirming previous studies (Hunter, 1992; Hunter *et al.*, 1993). The absence of sedative side-effects is an advantage of guanfacine when used clinically as an antihypertensive (Nami *et al.*, 1983). Absence of obvious CNS effects of guanfacine was surprising because guanfacine is well characterized as a centrally acting sympathoinhibitory and highly selective α_2-adrenoceptor agonist (Scholtysik, 1992). The reason for the absence of sedative effects of guanfacine, which is known to cross the blood–brain-barrier in rats, rabbits and dogs (Scholtysik, 1992), is not clear. Nothing is known on the penetration into the brain of cattle. However, sedation is thought to be mediated by α_2-adrenoceptors within the CNS as α_2-antagonists, such as tolazoline, inhibit xylazine-induced sedation in steers (Roming, 1984). Growth hormone secretion, too, is stimulated by α_2-agonists in the pituitary (Thomas *et al.*, 1994; Makara *et al.*, 1995; West *et al.*, 1997). In our study xylazine drastically increased GH plasma levels, whereas guanfacine had no effect. This cannot be explained based on present knowledge. However, in humans guanfacine enhanced GH release (Brown *et al.*, 1985). Assuming that guanfacine passes the bovine blood–brain-barrier, it can only be speculated that different subtypes of α_2-adrenoceptors are the target for the different ligands. There is

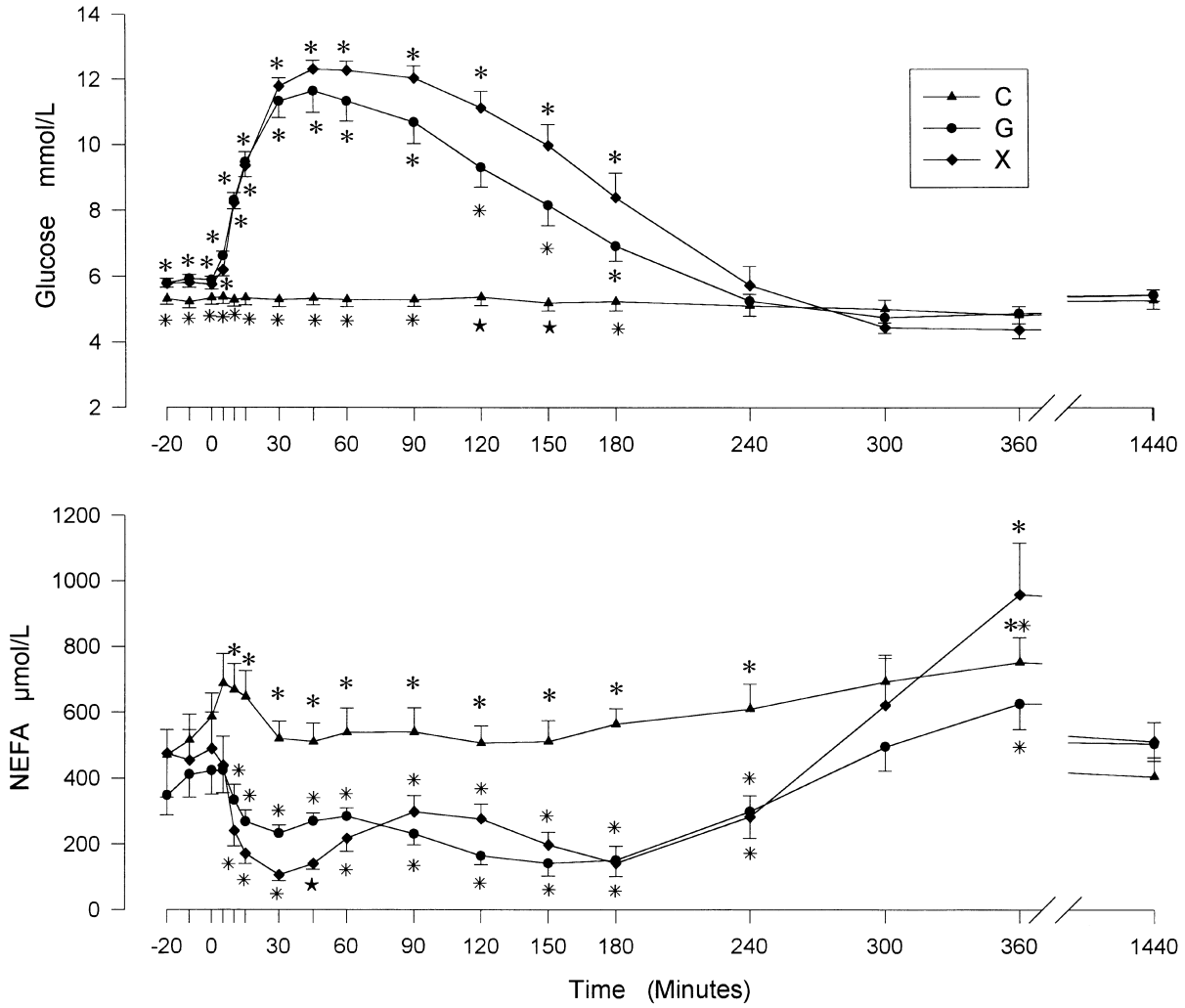

Fig. 5. Plasma levels of glucose and non esterified fatty acids (NEFA) in calves treated with xylazine (◆) or guanfacine (●) or sodium chloride solution (▲) at time zero. For details see legend to Fig. 1.

substantial variability between the affinity values determined for agonists (Hieble & Ruffolo, 1996). As stated in the introduction, guanfacine (MacLennan *et al.*, 1997) and xylazine (Smith *et al.*, 1995) exert affinity primarily to α_{2A}- and α_{2D}-adrenoceptors, respectively. Nothing is known on the distribution of these receptor subtypes in the bovine CNS with the exception of the pineal gland, which contains different α_{2D}-adrenoceptors (Simonneaux *et al.*, 1991), but the availability of ligands for the different α_2-adrenoceptor subtypes (MacKinnon *et al.*, 1992; Trendelenburg *et al.*, 1993, 1996; Bylund *et al.*, 1997) may help to clear the situation. It should also be mentioned that guanfacine has no affinity to imidazoline I_1 receptors in contrast to clonidine (Buccafusco *et al.*, 1995). The affinity of xylazine to these receptors mediating CNS sympathoinhibition (see Buccafusco *et al.*, 1995; Takada *et al.*, 1997) has to our knowledge not yet been investigated.

Decreases of the heart rate and of plasma catecholamine levels may be CNS-or peripherally-mediated. For many α_2-adrenoceptor agonists, including xylazine and guanfacine, central as well as peripheral (mediated *via* presynaptic receptors) sympathoinhibitory actions have been described (Brown *et al.*, 1985; Rump *et al.*, 1991; Hill *et al.*, 1993; Smith *et al.*, 1995; Schwartz, 1997). These actions cause lowering in heart rate and plasma catecholamine levels as found in this study and confirming previous results in steers (Hunter *et al.*, 1993; Gazzola *et al.*, 1995) and in humans (Nami *et al.*, 1983; Brown *et al.*, 1985).

Hyperglycaemia and initial inhibition of insulin secretion were seen in other species and were likely primarily peripheral α_2-agonistic effects (Blum, 1984; Spiers *et al.*, 1990; Sillence *et al.*, 1993; Miaron *et al.*, 1994). Both effects were measured in this study after xylazine and guanfacine administration. Inhibition of lipolysis *in vitro* and a decrease of plasma NEFA levels by some, but not all α-adrenoceptor agonists, has been documented in various species, including cattle (Blum *et al.*, 1978, 1982; Berlan *et al.*, 1980; Fain & Garcia-Sainz, 1983; Chilliard & Flechet, 1988). The secondary rise of insulin levels, as also seen by Cryer (1982), was likely the consequence of hyperglycaemic effects by xylazine and guanfacine.

The depressive effects on O_2 consumption, seen with both xylazine and guanfacine, were likely also the result of mixed

central and peripheral actions. These effects were in accordance with previous studies (Hunter, 1992; Gazzola *et al.*, 1993; Hunter *et al.*, 1993;).

In conclusion, our results demonstrate that the chemically different α_2-adrenoceptor agonists, xylazine and guanfacine, elicit qualitatively different effects or lack pharmacological actions mediated within the CNS of calves, whereas their peripheral actions seem to be quite similar. Based on present knowledge it cannot be decided to what extent pharmacokinetic properties (ability to cross the bovine blood–brain-barrier) or molecular properties (subtype α_2-adrenoceptor affinity) are responsible for the different effects of guanfacine and xylazine. In accordance with Hunter (1992) and Hunter *et al.* (1993) it is concluded that other indications, such as metabolic energy saving, independent from sedative and analgesic effects, may be an innovative use of certain α_2-agonists. Before interpretations on the mode of action can be given, the α_2-adrenoceptor subtype profile in the bovine brain regions, responsible for specific effects, should be investigated.

ACKNOWLEDGMENTS

The excellent technical assistance of Mr J. Lis, Inst. of Pharmacology and of Mrs. Claudine Morel and Mrs. Yolanda Zbinden, Div. of Nutrition Pathology, is gratefully acknowledged. Results of the present study were included in the thesis for Dr med. vet. of F. Regli, supervised by Dr A. Wüthrich and Dr B. Gassner and the authors wish to thank them for their invaluable help.

REFERENCES

Berlan, M., Lafontan, M. & Tran, L.D. (1980) La lipolyse adrénergique du tissu adipeux humain: propriétés et rôle physiologique des alpha récepteurs. *Journal of Physiology*, **76**, 133–146.

Blum, J.W. (1984) Insulin suppressive effects of aminotetralin analogs and of dopamine. *European Journal of Pharmacology*, **105**, 239–244.

Blum, J.W. & Flueckiger, N. (1988) Early metabolic and endocrine effects of perorally administered β-adrenoceptor agonists in calves. *European Journal of Pharmacology*, **151**, 177–187.

Blum, J.W., Froehli, D. & Kunz, P. (1982) Effects of catecholamines on plasma free fatty acids in fed and fasted cattle. *Endocrinology*, **110**, 452–456.

Blum, J.W., Guillebeau, A., Binswanger, U., Kunz, P., Da Prada, M. & Fischer, J.A. (1978) Effects of alpha-adrenergic stimulation and blockade on plasma parathyroid hormone concentrations in cows. *Acta Endocrinologica (Kbh)*, **88**, 535–543.

Brown, M.J., Struthers, A.D., Silvio, L.D., Yeo, T., Ghatei, M. & Burrin, J. (1985) Metabolic and haemodynamic effects of alpha-2-adrenoceptor stimulation and antagonism in man. *Clinical Sciences*, **68**, 137S–139S.

Bruckmaier, R.M. & Blum, J.W. (1992) Responses of calves to treadmill exercise during beta-adrenergic agonist administration. *Journal of Animal Science*, **70**, 2809–2821.

Buccafusco, J.J., Lapp, C.A., Westbrooks, K.L. & Ernsberger, P. (1995) Role of medullary I_1-imidazoline and α_2-adrenergic receptors in the antihypertensive responses evoked by central administration of clonidine analogs in conscious spontaneously hypertensive rats. *The Journal of Pharmacology and Experimental Therapeutics*, **273**, 1162–1171.

Bylund, D.B., Eikenberg, D.C., Hieble, J.P., Langer, S.Z., Lefkowitz, R.J., Minneman, K.P., Molinoff, P.B., Ruffolo, R.R. Jr & Trendelenburg, A.U. (1994) IV international union of pharmacology nomenclature of adrenoceptors. *Pharmacological Reviews*, **46**, 121–136.

Bylund, D.B., Hass, N.A., Cerutis, D.R. & Blaxall, H.S. (1995) Characterization of alpha-2 adrenergic receptor subtypes. *Pharmacological Research Communications*, **6**, 87–90.

Bylund, D.B., Iversen, L.J., Matulka, W.J. & Chacko, D.M. (1997) Characterization of alpha-2D adrenergic receptor subtypes in bovine ocular tissue homogenates. *The Journal of Pharmacology and Experimental Therapeutics*, **281**, 1171–1177.

Chilliard, Y. & Flechet, J. (1988) Effets de la clonidine (alpha-2 agoniste) sur la lipolyse du tissu adipeux de bovin adulte, in vitro. *Reproduction, Nutrition et Développement*, **28**, 195–196.

Cryer, P.E. (1982) Clinical syndromes of autonomic failure. *Cardiovascular Research*, **16**, 370–376.

Fain, J.N. & Garcia-Sainz, J.A. (1983) Adrenergic regulation of adipocyte metabolism. *Journal of Lipid Research*, **24**, 945–966.

Ganhao, M.F., Hatting, J., Hurwitz, M.L. & Pitts, N.I. (1991) Evaluation of a simple plasma catecholamine extraction procedure prior to high-performance liquid chromatography and electrochemical detection. *Journal of Chromatography*, **564**, 55–56.

Gazzola, C. (1993) α_2-adrenoceptor-mediated effects on resting energy expenditure. *International Journal of Obesity*, **17**, 637–641.

Gazzola, C. (1995) The effect of the α_2-adrenoceptor agonist, guanfacin, on energy expenditure, intake and deposition in rats. *Comparative Biochemistry and Physiology*, **112C**, 29–34.

Gazzola, C., Magner, T., Berger, K.T. & Hunter, R.A. (1993) Effects of guanfacin and nitroprusside on the haemodynamics and oxygen consumption of Brahman steers. *Comparative Biochemistry and Physiology*, **106C**, 443–448.

Gazzola, C., Magner, T., Lisle, A.T. & Hunter, R.A. (1995) Effects of α-adrenoceptor agonists and antagonists on metabolic rate in cattle. *Comparative Biochemistry and Physiology*, **111A**, 73–77.

Gerlo, E. & Malfait, R. (1985) HPLC-Assay of free norepinephrine, epinephrine, dopamine, vanillylmandelic acid and homovanilic acid. *Journal of Chromatography*, **343**, 9–20.

Harrison, J.K., Pearson, W.R. & Lynch, K.R. (1991) Molecular characterization of α_1- and α_2-adrenoceptors. *Trends in Pharmacological Sciences*, **12**, 62–67.

Hieble, J.P. & Ruffolo, R.R. (1996) Subclassification and nomenclature of α_1- and α_2-adrenoceptors. *Progress in Drug Research*, **47**, 81–123.

Hill, C.E., Powis, D.A. & Hendry, I.A. (1993) Involvement of pertussis toxin-sensitive and -insensitive mechanisms in α-adrenoceptor modulation of noradrenaline release from rat sympathetic neurones in tissue culture. *British Journal of Pharmacology*, **110**, 281–288.

Horvai, G. & Pungor, E. (1989) Electrochemical detectors in HPLC and ion chromatography. *CRC Critical Reviews in Analytical Chemistry*, **21**, 1–28.

Hostettler-Allen, R.L., Tappy, L. & Blum, J.W. (1994) Insulin resistance, hyperglycemia, and glucosuria in intensively milk-fed calves. *Journal of Animal Science*, **72**, 160–173.

Hunter, R.A. (1992) The effect of the α_2-adrenergic agonist, guanfacin, on the energy metabolism of steers fed on low-quality-roughage diets. *British Journal of Nutrition*, **67**, 337–343.

Hunter, R.A., Sillence, M.N., Gazzola, C. & Spiers, W.G. (1993) Increasing annual growth rates of cattle by reducing maintenance energy requirements. *Australian Journal of Agricultural Research*, **44**, 579–595.

Kleinlogel, H., Scholtysik, G. & Sayers, A.C. (1975) Effects of clonidine and BS 100–141 on the ECG sleep pattern in rats. *European Journal of Pharmacology*, **33**, 156–163.

Kroneberg, G., Oberdorf, A., Hoffmeister, F. & Wirth, W. (1967) Zur Pharmakologie von 2-(2,6-Dimethylphenylamino)-4H–5,6–dehydro–1,3–thiazin (Bayer 1470), eines Hemmstoffes adrenergischer und

cholinergisher Neurone. *Naunyn – Schmiedeberg's Archives of Pharamcology*, **256**, 257–280.

Kümper, H. (1989) Nebenwirkungen von α_2-Sympathomimetika beim Rind. *Vet*, **9**, 6–13.

Kyles, A.E., Waterman, A.E. & Livingston, A. (1993) The spinal antinociceptive activity of the α_2-adrenoceptor agonist, xylazine in sheep. *British Journal of Pharmacology*, **108**, 907–913.

MacDonald, E., Kobilka, B.K. & Scheinin, M. (1997) Gene targeting-homing in on α_2-adrenoceptor-subtype function. *Trends in Pharmacological Sciences*, **18**, 211–219.

MacKinnon, A.C., Kilpatrick, A.T., Kenny, B.A., Spedding, M. & Brown, C.M. (1992) [^{3}H]-RS15385–197, a selective and high affinity radioligand for α_2-adrenoceptors: implications for receptor classification. *British Journal of Pharmacology*, **106**, 1011–1018.

MacLennan, S.J., Luong, L.A., Jasper, J.R., To, Z.P. & Eglen, R.M. (1997) Characterization of α_2-adrenoceptors mediating contraction of dog saphenous vein: identity with the human α_{2A} subtype. *British Journal of Pharmacology*, **121**, 1721–1729.

Makara, G.B., Kiem, D.T. & Vizi, E.S. (1995) Hypothalamic α_{2A}-adrenoceptors stimulate growth hormone release in the rat. *European Journal of Pharmacology*, **287**, 43–48.

Miaron, J.O.O., Christopherson, R.J., Hardin, R.T., Mosenthin, R. & Cosgrove, S.J. (1994) The effect of the α_2-adrenoceptor agonist guanfacine on heat production of steers in different thermal environments. *Proceedings of the Society of Nutrition Physiology*, **3**, 313.

Nami, R., Bianchini, C., Fiorella, G., Chierichetti, S.M. & Gennari, C. (1983) Comparison of effects of guanfacine and clonidine on blood pressure, heart rate, urinary catecholamines, and cyclic nucleotides during and after administration to patients with mild to moderate hypertension. *Journal of Cardiovascular Pharmacology*, **5**, 546–551.

Roming, L.G.P. (1984) Tolazolin als Xylazin-Antagonist beim Rind. *Deutsche Tierärztliche Wochenschrift*, **91**, 154–157.

Ruffolo, R.R., Nichols, A.J., Stadel, J.M. & Hieble, J.P. (1993) Pharmacological and therapeutic applications of α_2-adrenoceptor subtypes. *Annual Review of Pharmacology and Toxicology*, **32**, 243–279.

Rump, L.Ch., Ruff, G., Wolk, V. & Schollmeyer, P. (1991) α_2-Adrenoceptor activation inhibits noradrenaline release in human and rabbit isolated renal arteries. *European Journal of Pharmacology*, **196**, 277–283.

SAS (1989) SAS user's guide: statistics. SAS Inst. Inc., Cary, NC.

Scholtysik, G. (1992) Centrally acting antihypertensive α_2-adrenoceptor agonists. In *Progress in Hypertension.* Vol. 2. Eds Saito, H., Minami, M. & Parvez, S.H. pp. 257–268. VSP, Utrecht, The Netherlands.

Schwartz, D.D. (1997) Activation of alpha-2 adrenergic receptors inhibits norepinephrine release by a pertussis toxin-insensitive pathway independent of changes in cytosolic calcium in cultured rat sympathetic neurons. *The Journal of Pharmacology and Experimental Therapeutics*, **282**, 248–255.

Sillence, M.N., Tudor, G.D., Matthews, M.L. & Lindsay, D.B. (1993) Effects of the α_2-adrenoceptor agonist guanfacine on growth and thermogenesis in mice. *Journal of Animal Science*, **70**, 3429–3434.

Simonneaux, V., Ebadi, M. & Bylund, D.B. (1991) Identification and characterization of different α_{2D}-adrenergic receptors in bovine pineal gland. *Molecular Pharmacology*, **40**, 235–241.

Smith, K., Gavin, K. & Docherty, J.R. (1995) Investigation of the subtype of α_2-adrenoceptor mediating prejunctional inhibition of cardioacceleration in the pithed rat heart. *British Journal of Pharmacology*, **115**, 316–320.

Spiers, W.G., Sillence, M.N. & Lindsay, D.B. (1990) α_2-agonist-induced effects on growth in rats. *Proceedings of the Nutrition Society of Australia*, **15**, 172.

Takada, K., Hayashi, Y., Kamibayashi, T., Mammoto, T., Yamatodani, A., Kitamura, S. & Yoshiya, I. (1997) The involvement of pertussis toxin-sensitive G proteins in the post receptor mechanism of central I_1-imidazoline receptors. *British Journal of Pharmacology*, **120**, 1575–1581.

Thomas, G.B., Scott, C.J., Cummins, J.T. & Clarke, I.J. (1994) Adrenergic regulation of growth hormone secretion in the ewe. *Domestic Animal Endocrinology*, **11**, 187–195.

Trendelenburg, A.-U., Limberger, N. & Starke, K. (1993) Presynaptic α_2-autoreceptors in brain cortex: α_{2D} in the rat and α_{2A} in the rabbit. *Naunyn-Schmiedeberg's Archives of Pharmacology*, **348**, 35–45.

Trendelenburg, A.-U., Wahl, C.A. & Starke, K. (1996) Antagonists that differentiate between α_{2A}- and α_{2D}-adrenoceptors. *Naunyn-Schmiedeberg's Archives of Pharmacology*, **353**, 245–249.

West, C.R., Gaynor, P.J., Lookingland, K.J. & Tucker, H.A. (1997) Regulation of growth hormone-releasing hormone and somatostatin from perifused bovine hypothalamic slices. I. α_2-adrenergic receptor regulation. *Domestic Animal Endocrinology*, **14**, 334–348.

Zimmerli, U.V. & Blum, J.W. (1990) Acute and longterm metabolic, endocrine, respiratory, cardiac and skeletal muscle activity changes in response to perorally administered β-adrenoceptor agonists in calves. *Journal of Animal Physiology and Animal Nutrition*, **63**, 157–172.