Effects of Atipamezole, an α_2 -Adrenergic Antagonist, and Somatostatin on Xylazine-Induced Growth Hormone Release in Calves

ETSUKO KASUYA, KOICHI HODATE*, MITSUTO MATSUMOTO*, MINORU SAKAGUCHI*, TSUTOMU HASHIZUME, AND SHIGETO KANEMATSU

Laboratory of Animal Reproduction, Faculty of Agriculture, Iwate University, Iwate 020, and

* Tohoku National Agricultural Experiment Station, Iwate 020-01, Japan

Abstract. In order to clarify the mechanism of xylazine-induced GH release, we investigated the effects of atipamezole, a selective α_2 -adrenergic antagonist, and somatostatin (SRIF) on xylazine-stimulated GH release in calves. Xylazine injection (0.30 mg/kg BW, iv) induced a rapid increase in the GH concentration. When atipamezole was used in combination with xylazine, it blunted the increase in the plasma GH concentration induced by the xylazine injection. The GH levels at 15–50 min after the simultaneous injection of xylazine and atipamezole were significantly ($P<0.05$) lower than the corresponding values in the animals given xylazine alone. The area under the GH response curve for 120 min after the simultaneous injection of xylazine and atipamezole was significantly ($P<0.05$) smaller than that for the xylazine alone. A series of five intravenous injections of 1 mg of SRIF at 10-min intervals also blunted xylazine-stimulated GH release. Atipamezole partially suppressed xylazine-induced hyperglycemia, but SRIF completely suppressed the hyperglycemia for the first 60 min after the xylazine injection and the suppression by SRIF was stronger than that by atipamezole. On the other hand, both atipamezole and SRIF failed to blunt xylazine-induced hypoinsulinemia. The present results suggest that xylazine stimulates GH release via the α_2 -adrenergic pathway in cattle, but the mechanism of xylazine-induced hyperglycemia remains to be determined.

Key words: Xylazine, Atipamezole, GH, Calves

(Endocrine Journal 43: 551–556, 1996)

GH secretion is mainly regulated by two hypothalamic factors, GH-releasing hormone (GHRH) and somatostatin (SRIF) [1]. Recent evidence has suggested that other factors such as neurotransmitters may be involved in GH release by modifying the hypothalamic factors [2].

Xylazine hydrochloride is a sedative analgesic muscle relaxant agent which was first synthesized in Germany in 1962 [3]. It has been widely em-

ployed for veterinary clinical use and has proven to be relatively safe when used alone or in combination with other anesthetics [4]. This agent has been known to stimulate GH release in dogs [5]. We also reported that xylazine injection resulted in a GH increase in cattle [6] as in dogs [5]. Xylazine is also known to induce hypoinsulinemia and hyperglycemia in ruminants [6–11] but, as far as we know, no mechanism of xylazine-induced GH release has ever been reported in any animal species. It is likely that α_2 -adrenergic receptors regulate the response of GH to xylazine injection, since xylazine-induced sedation is mediated by α_2 -adrenoceptors [4].

Atipamezole hydrochloride (4-(2-ethyl-2,3-dihy-

Received: December 14, 1995

Accepted: May 29, 1996

Correspondence to: Prof. Shigeto KANEMATSU, Laboratory of Animal Reproduction, Faculty of Agriculture, Iwate University, Ueda 3, Morioka 020, Japan

dro-1H-inden-2-yl)-1H-imidazole hydrochloride) is a potent and selective α_2 -adrenergic receptor antagonist which reverses the sedative effect produced by the α_2 -adrenergic agonist medetomidine [12, 13]. Atipamezole enhances the release and turnover of the neurotransmitter noradrenaline in both the central and peripheral nervous systems.

In the present study, we investigated the effect of atipamezole on GH release in calves, to test whether xylazine induced GH release via the α_2 -adrenergic pathway. In addition to GH, we determined the plasma glucose and insulin concentrations in order to clarify the relationship among the induced GH release, hypoinsulinemia and hyperglycemia. Furthermore, the effect of atipamezole on xylazine-induced GH release was compared with that of SRIF.

Materials and Methods

Three castrated male calves and one female with a 223.9 ± 5.4 kg body weight (BW) were used. They were individually fed corn silage and concentrate from 0830 h to 0930 h and from 1700 h to 1800 h and supplied water *ad libitum*. Xylazine hydrochloride (Celactal, Bayer, Germany) was injected intravenously (0.30 mg/kg BW) at 1100 h. Atipamezole hydrochloride (Antisedan, Farnos, Turku, Finland) was administered intramuscularly (30 μ g/kg BW) at the same time. Somatostatin-14 (SRIF, Peptide Institute, Inc., Osaka, Japan) was injected intravenously (5 mg/head, divided into five injections at 10-min intervals). The injection of SRIF was started at 1100 h. As vehicle controls, sterilized saline was injected into four calves. A catheter was inserted acutely into the jugular vein 60 min before the experiments and the agents were injected via this indwelling catheter. Blood samples were collected via the catheter under unstressful conditions. Blood samples were collected at 5-min (for 30 min after the xylazine, atipamezole or saline injection), 10-min (for 30–60 min after the injection), 15-min (for 60–120 min after the injection) and 30-min (for 120–360 min after the injection) intervals. The blood samples were collected into heparinized tubes and immediately kept on ice. The plasma was separated by centrifugation and stored at -20°C until hormone

determinations.

Plasma GH concentration was measured by a double antibody radioimmunoassay (RIA) [14]. Coefficients of variation (CV) of the intra- and interassay of the RIA were 7% and 11%, respectively. The glucose concentration in plasma was determined with a GOD Kit (Glucose-HA Test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and an Autoanalyzer 705 (Hitachi, Japan). The plasma insulin level was measured by with a commercially available RIA kit (Insulin Radioimmunoassay Kit, Eiken Chemical Co., Ltd., Tokyo, Japan). The intra-assay CV of insulin RIA was 3%.

As an index of the amount of GH released in response to the agents or saline, the area under the GH response curve (AUC) for 120 min after the injection was calculated. Data represent the mean $\pm$ SEM. Statistical significance of differences was determined by Student's *t*-test or Cochran-Cox test after analysis of uniformity of variance by Bartlett's test [15].

Results

The maximum duration of sedation caused by xylazine was 150 min in the present study. As shown in Fig. 1, saline injections did not alter basal GH concentrations. On the other hand, xylazine injections (0.30 mg/kg BW, iv) significantly ($P < 0.05$) stimulated GH release. The level of GH began to rise 5 min after the injection. After the peak level was reached at 25 min after the administration, GH level began to decrease and it returned to the level of the controls 1.5 h after the xylazine injection. Injection of atipamezole at a dose of 30 μ g/kg BW did not alter the basal GH concentration when the drug was used alone. On the other hand, when xylazine (0.30 mg/kg BW) and atipamezole (30 μ g/kg BW) were injected simultaneously, atipamezole blunted the release of GH induced by the xylazine. The GH levels at 15–50 min after the simultaneous injection of xylazine and atipamezole were significantly ($P < 0.05$) lower than the corresponding values in the animals given the xylazine alone. SRIF also blunted xylazine-induced GH release. The GH concentrations at 15, 20 and 25 min after the xylazine injection were significantly ($P < 0.05$) lower than the

Fig. 1. Plasma concentrations of GH after injections of xylazine alone ($-\diamond-$, 0.30 mg/kg BW, iv), atipamezole alone ($-\bullet-$, 30 μ g/kg BW, im), xylazine + atipamezole ($-\triangle-$) and xylazine + somatostatin-14 (SRIF, $-\nabla-$, 5 mg/head, 1 mg/each injection, iv) or saline ($-\circ-$) in calves. The solid arrow indicates time of injections for xylazine, atipamezole, xylazine + atipamezole, or saline. Open arrows indicate time of serial injections of SRIF. * $P < 0.05$ compared with the corresponding values for the saline injected control group. # $P < 0.05$ compared with the corresponding values for the xylazine alone injected group. Each point represents the mean $\pm$ SEM for 4 calves.

Fig. 2. Area under GH response curve (AUC) for 120 min after injections of xylazine alone, atipamezole alone, xylazine + atipamezole, xylazine + SRIF or saline in calves. * $P < 0.05$ as compared with saline injected control group. # $P < 0.05$ as compared with xylazine alone injected group. Each value represents the mean $\pm$ SEM for 4 calves.

corresponding values in the animals injected with xylazine alone.

Figure 2 shows the AUC for 120 min after each treatment. The AUC for xylazine alone was significantly ($P < 0.05$) greater than that for controls. And the AUC for atipamezole with xylazine was significantly ($P < 0.05$) smaller than that for xylazine alone. There was no significant difference between AUCs for atipamezole with xylazine and for SRIF with xylazine.

The plasma concentration of glucose was significantly ($P < 0.05$) enhanced by 0.30 mg/kg BW of xylazine (Fig. 3). This increase in glucose was maintained for 360 min after the injection. Atipamezole had no effect on the plasma glucose level when it was used alone. When atipamezole was administered in combination with xylazine, the agent inhibited the response of plasma glucose to xylazine injection. The values at 30–50 min after the injections of xylazine together with atipame-

Fig. 3. Plasma concentrations of glucose after injections of xylazine alone ($-\diamond-$), atipamezole alone ($-\bullet-$), xylazine + atipamezole ($-\triangle-$) and xylazine + SRIF ($-\nabla-$) or saline ($-\circ-$) in calves. The solid arrow indicates time of injections for xylazine, atipamezole, xylazine + atipamezole, or saline. Open arrows indicate the times of serial injections of SRIF. * $P < 0.05$ compared with the corresponding values for the saline injected control group. # $P < 0.05$ compared with the corresponding values for the xylazine alone injected group. Each point represents the mean $\pm$ SEM for 4 calves.

Fig. 4. Plasma concentrations of insulin after injections of xylazine alone ($\diamond$), atipamezole alone ($\bullet$), xylazine + atipamezole ($\triangle$) and xylazine + SRIF ($\blacktriangledown$) or saline ($\circ$) in calves. The solid arrow indicates time of injections for xylazine, atipamezole, xylazine + atipamezole, or saline. Open arrows indicate the times of serial injections of SRIF. * $P < 0.05$ compared with the corresponding values for the saline injected control group. Each point represents the mean $\pm$ SEM for 4 calves.

zole were significantly ($P < 0.05$) lower than the corresponding values for calves injected with xylazine alone. Serial injections of SRIF completely suppressed xylazine-induced hyperglycemia for the first 60 min after the xylazine injection. The suppression by SRIF was stronger than that by atipamezole for the doses used. The glucose values at 10–60 min after the injection of xylazine were significantly ($P < 0.05$) lower than the corresponding values for calves treated with xylazine alone.

As shown in Fig. 4, xylazine decreased plasma insulin concentrations in calves. The insulin concentrations at 30, 50, 75 and 90 min after the xylazine injection were significantly ($P < 0.05$) lower than the corresponding values for saline-injected control animals. Atipamezole and SRIF failed to alter this response in insulin. A rebound increase in insulin concentrations was observed 5 and 6 h after the injection in three groups treated with xylazine alone, xylazine plus atipamezole, and xylazine plus SRIF.

Discussion

The present results clearly demonstrated that injection of an α_2 -adrenergic antagonist, atipamezole,

completely blocked the response of GH to xylazine injection, which indicates that xylazine stimulates GH release via an α_2 -adrenergic pathway in calves. It has been reported that xylazine induced GH release in calves [6] and dogs [5]. Xylazine-induced sedation is mediated by α_2 -adrenoceptors [4]. Alpha₂-adrenergic receptors therefore play an important role in GH response to xylazine.

It has been demonstrated that the GH-releasing action of the α_2 -adrenergic mechanism was mediated through the stimulation of GHRH in the rat [16–19]. On the other hand, there are some reports which proposed that the stimulatory effect of clonidine, an α_2 -adrenoceptor agonist, on GH release is mainly exerted by inhibiting the hypothalamic SRIF release in rats [20–22] and man [23]. But the study by Magnan *et al.* in sheep [24] has demonstrated that clonidine acts centrally to stimulate GH and that this effect is mediated through changes in GHRH release into hypophyseal portal blood.

It is therefore possible that xylazine induces GH release via GHRH release through stimulation of α_2 -adrenergic receptors in calves. In the present study, intravenous injections of SRIF blunted xylazine-induced GH release in calves. This suggested that exogenous SRIF inhibited xylazine-induced GH release at the pituitary level, but since SRIF can inhibit release of GHRH [25, 26], the increased GHRH release induced by xylazine might have been partially inhibited by SRIF in the present study.

We have assumed several mechanisms for xylazine-induced hyperglycemia, which include activation of α_2 -adrenergic receptors, increased hepatic glucose production, increased glucagon release and hypoinsulinemia. Since the hyperglycemia was inhibited by another α_2 -antagonist, yohimbine, in both cattle [7] and dogs [27], it is suggested that the hyperglycemia was caused by activating α_2 -adrenergic receptors. It has been reported that intravenous xylazine treatments increased glucose production directly on the liver in cattle [11]. In addition, xylazine has been reported to increase glucagon release from the pancreas in sheep [8]. It is well known that SRIF inhibits glucagon release from the pancreas [28]. In the present study, SRIF completely inhibited xylazine-induced hyperglycemia for the first 60 min after the xylazine injection. The increased glucagon

gon release may be involved in the hyperglycemia. Moreover, the inhibition of xylazine-induced hyperglycemia in calves by exogenous SRIF was stronger than that by atipamezole. In the present study, although the maximum duration of xylazine-induced hypoinsulinemia and the sedative effect of xylazine was 240 min and 150 min, respectively, xylazine-induced hyperglycemia continued for 360 min. On the other hand, both the atipamezole and SRIF failed to blunt xylazine-induced hypoinsulinemia. It is therefore assumed

that the most important factor in xylazine-induced hyperglycemia would be an increase in glucagon stimulated by the xylazine, but this hypothesis remains to be confirmed, since glucagon levels were not determined in the present study.

In conclusion, the present results suggested that xylazine stimulates GH release via an α_2 -adrenergic pathway in cattle, but the mechanism of xylazine-induced hyperglycemia remains to be determined.

References

1. Frohman LA, Downs TR, Chomczynski P (1992) Regulation of growth hormone secretion. *Frontiers in Neuroendocrinology* 13: 344–405.
2. Devesa J, Lima L, Tresguerres JAF (1992) Neuroendocrine control of growth hormone secretion in humans. *Trends Endocrinol Metab* 3: 175–183.
3. Knight AP (1980) Xylazine. *J Am Vet Med Assoc* 176: 454–455.
4. Greene SA, Thurmon JC (1988) Xylazine—A review of its pharmacology and use in veterinary medicine. *J Vet Pharmacol Therap* 11: 295–313.
5. Hampshire J, Altszuler N (1981) Clonidine or xylazine as provocative tests for growth hormone secretion in the dog. *Am J Vet Res* 42: 1073–1076.
6. Kasuya E, Hodate K, Matsumoto M, Sakaguchi M, Hashizume T, Kanematsu S (1996) The effects of xylazine on plasma concentrations of growth hormone, insulin-like growth factor-I, glucose and insulin in calves. *Endocr J* 43: 145–149.
7. Hsu WH, Hummel SK (1981) Xylazine-induced hyperglycemia in cattle: A possible involvement of α_2 -adrenergic receptors regulating insulin release. *Endocrinology* 109: 825–829.
8. Brockman RP (1981) Effect of xylazine on plasma glucose, glucagon and insulin concentrations in sheep. *Res Vet Sci* 30: 383–384.
9. Eichner RD, Prior RL, Kvasnicka WG (1979) Xylazine-induced hyperglycemia in beef cattle. *Am J Vet Res* 40: 127–129.
10. Symonds HW (1976) The effect of xylazine upon hepatic glucose production and blood flow rate in the lactating dairy cow. *Vet Rec* 99: 234–236.
11. Symonds HW, Mallinson CB (1978) The effect of xylazine and xylazine followed by insulin on blood glucose and insulin in the dairy cow. *Vet Rec* 102: 27–29.
12. Wong WC, Gluchowski C (1995) A concise synthesis of atipamezole. *Synthesis* 1995(2): 139–140.
13. Nishimura R, Kim H, Matsunaga S, Sakaguchi M, Sasaki N, Tamura H, Takeuchi A (1992) Antagonism of medetomidine sedation by atipamezole in pigs. *J Vet Med Sci* 54: 1237–1240.
14. Johke T (1978) Effect of TRH on circulating growth hormone, prolactin and triiodothyronine levels in the bovine. *Endocrinol Japon* 25: 19–26.
15. Wakabayashi K (1984) Arrangement of Experimental Data. Baihukan, Tokyo, 44–57, 90–133.
16. Katakami H, Kato Y, Matsushita N, Imura H (1984) Effects of neonatal treatment with monosodium glutamate on growth hormone release induced by clonidine and prostaglandin E_1 in conscious male rats. *Neuroendocrinology* 38: 1–5.
17. Miki N, Ono M, Shizume K (1984) Evidence that opiate and α -adrenergic mechanisms stimulate rat growth hormone release via growth hormone-releasing factor (GRF). *Endocrinology* 114: 1950–1952.
18. Kabayama Y, Kato Y, Murakami Y, Tanaka H, Imura H (1986) Stimulation by alpha-adrenergic mechanisms of the secretion of growth hormone-releasing factor (GRF) from perfused rat hypothalamus. *Endocrinology* 119: 432–434.
19. Gil-Ad I, Laron Z, Koch Y (1991) Effect of acute and chronic administration of clonidine on hypothalamic content of growth hormone-releasing hormone and somatostatin in the rat. *J Endocrinol* 131: 381–385.
20. Lima L, Arce V, Tresguerres JAF, Devesa J (1993) Clonidine potentiates the growth hormone (GH) response to GH releasing-hormone in norepinephrine synthesis-inhibited rats: Evidence for an alpha-2-adrenergic control of hypothalamic release of somatostatin. *Neuroendocrinology* 57: 1155–1160.
21. Lanzi R, Lapointe M, Gurd W, Tannenbaum GS (1994) Evidence for a primary involvement of somatostatin in clonidine-induced growth hormone release in conscious rats. *J Endocrinol* 141: 259–266.
22. Arce V, Barros MG, Vara E, Lima L, Tresguerres JAF, Devesa J (1995) Clonidine potentiates the

- growth hormone response to a growth hormone releasing hormone challenge in hypothalamic growth hormone releasing hormone deficient rats. *Neuroendocrinology* 61: 552–558.
23. Devesa J, Diaz MJ, Tresguerres JAF, Arce V, Lima L (1991) Evidence that α_2 -adrenergic pathways play a major role in growth hormone (GH) neuroregulation: α_2 -adrenergic agonism counteracts the inhibitory effect of muscarinic cholinergic receptor blockade on the GH response to GH-releasing hormone, while α_2 -adrenergic blockade diminishes the potentiating effect of increased cholinergic tone on such stimulation in normal men. *J Clin Endocrinol Metab* 73: 251–256.
24. Magnan E, Cataldi M, Guillaume V, Mazzocchi L, Dutour A, Razafindraibe H, Sauze N, Renard M, Oliver C (1994) Role of growth hormone (GH)-releasing hormone and somatostatin in the mediation of clonidine-induced GH release in sheep. *Endocrinology* 134: 562–567.
25. Miki N, Ono M, Shizume K (1988) Withdrawal of endogenous somatostatin induces secretion of growth hormone-releasing factor in rats. *J Endocrinol* 117: 245–252.
26. Magnan E, Cataldi M, Guillaume V, Conte Devolx B, Graziani N, Figaroli JC, Thomas F, Chihara K, Oliver C (1992) Acute changes in growth hormone-releasing hormone secretion after injection of BIM 23014, a long acting somatostatin analog, in rats. *Life Sci* 51: 831–838.
27. Hsu WH (1988) Yohimbine increases plasma insulin concentrations and reverses xylazine-induced hypoinsulinemia in dogs. *Am J Vet Res* 49: 242–244.
28. Muller EE (1987) Neural control of somatotrophic function. *Physiol Rev* 67: 962–1053.