

α_2 -Adrenergic Modulation of Glucagon and Insulin Secretions in Sheep

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ODA, S., FUJIMURA, H., SASAKI, Y. and OHNEDA, A. *α_2 -Adrenergic Modulation of Glucagon and Insulin Secretions in Sheep.* Tohoku J. Exp. Med., 1991, 163 (2), 101-110 — To investigate the effects of α_2 -adrenergic receptors on the secretions of pancreatic glucagon and insulin, clonidine, midaglizole and yohimbine were intravenously administered in four conscious sheep. Clonidine infusion at a dosage of 1.0 nmol/kg/min produced hyperglucagonemia, hypoinsulinemia and hyperglycemia. Midaglizole or yohimbine was infused for 30 min, at doses of 5, 10 and 50 nmol/kg/min during the clonidine infusion. The highest yohimbine infusion (50 nmol/kg/min) blocked the clonidine-induced responses of glucagon, insulin and glucose. On the other hand, the midaglizole (50 nmol/kg/min) infusion brought about no statistical effect on the clonidine-induced responses of glucagon, insulin and glucose. The α_2 -adrenergic antagonistic effect of midaglizole was clearly less than that of yohimbine in the present experiments. It is concluded that the glucagon secretion is enhanced and the insulin release is inhibited by α_2 -adrenergic stimulation in conscious sheep. ——— clonidine; midaglizole; yohimbine; glucagon; insulin

Recently, it has been demonstrated that adrenergic subtype receptor mechanism modulates glucagon and insulin secretion in several species. Xylazine, an α_2 -adrenergic agonist, caused hyperglucagonemia, hypoinsulinemia and hyperglycemia in sheep (Muggaberg and Brockman 1982). Both phenylephrine, an α_1 -adrenergic agonist, and clonidine, an α_2 -adrenergic agonist, increased plasma glucagon and lowered plasma insulin in mouse (Skoglund et al. 1987). It has been shown that yohimbine, an α_2 -adrenergic antagonist, eliminated the glucagon response to epinephrine infusion in rats (Patel 1984), in rabbits (Knudtson 1984b) and in sheep (Oda et al. 1990). On the contrary, clonidine was ineffective on glucagon secretion in human (Ferlito et al. 1985), in rat islet cell (Schuit and Pipeleers 1986) and in rat pancreas (Filipponi et al. 1986). Furthermore, studies using metoprolol, a selective β_1 -adrenergic antagonist, suggest that the adrenergic stimulation of glucagon secretion was mediated through β_1 -receptors in rat pancreas (Gregorio et al. 1986). Thus it may be conceivable that the conflicting results concerning the modulation of glucagon secretion with adrenergic subtype

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receptor are attributed to the species differences or the differences of experimental design. In addition, our preliminary findings indicates that the α_2 -adrenergic subtype mechanism was more important in responses of glucagon and insulin than the α_1 -adrenergic subtype mechanism (Oda et al. 1990). Therefore, in the present experiment, we studied the effects of clonidine and the α_2 -adrenergic antagonists, yohimbine and midaglizole, on the secretory responses of glucagon and insulin in conscious sheep to obtain further information concerning the α_2 -adrenergic modulation in glucagon secretion.

MATERIALS AND METHODS

Animals

Four adult castrated male sheep weighing 42 to 47 kg were used. The sheep were housed in metabolic cages and offered 900 g of alfalfa pellets in a single meal at 5 p.m. Water was available continuously. At least 3 months before experiments began they had their left common carotid artery chronically placed in a loop of skin under general anesthesia with pentobarbital sodium (25 mg/kg). At least 1 week before experiments, a polyethylene catheter for infusion was inserted into the jugular vein through a hypodermic needle. The catheter was kept patent by daily flushing with a heparinized saline.

Experimental procedures

A catheter for blood sampling was inserted into the exteriorized carotid artery at least 1 hr before the experiment.

In the first experiment, in order to investigate the effect of the α_2 adrenergic agonist upon the secretions of glucagon and insulin from the pancreas, clonidine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA) dissolved in sterile saline was administered in a dosage of 1.0 nmol/kg/min for 180 min through the jugular catheter. The data were compared with those of saline infusion. In the second experiment, the α_2 -adrenergic antagonists, midaglizole (Daiichi Pharmaceutical Co., Ltd., Tokyo) and yohimbine hydrochloride (Sigma Chemical Co.) were administered through the jugular catheter during the clonidine infusion (1.0 nmol/kg/min). Midaglizole and yohimbine were infused for 30 min from 30 to 60 min and from 120 to 150 min, respectively, at doses of 0 (saline), 5, 10 and 50 nmol/kg/min. Midaglizole and yohimbine were infused at the same dosage. The four experiments were assigned for Latin square design (4 animals \times 4 doses of the α_2 -adrenergic antagonists). Each experiment was performed at 1-day intervals. The timing of blood samples is indicated in Figs. 1 and 2, respectively.

Analyses

Blood samples collected with heparinized syringes were immediately transferred into polyethylene test tubes cooled in ice water and centrifuged at 4°C. Each tube contained 0.05 mmoles benzamidine hydrochloride (Sigma Chemical Co.) as a proteolytic inhibitor per 1 ml blood. A portion of plasma was deproteinized with 10% (W/V) trichloroacetic acid, and the supernatant was stored at -20°C until glucose was determined by the glucose oxidase method (Huggett and Nixon 1957). Plasma immunoreactive glucagon (IRG) was determined by the radioimmunoassay (Ohneda et al. 1975) using dextran-coated charcoal and an antiserum G42-E, which was highly specific for the C-terminal portion of glucagon (Ohneda et al. 1979). Crystalline porcine glucagon was used as a standard (Lot 102067; Calbiochem, Los Angeles, CA, USA), and porcine [125 I]glucagon was purchased from New England Nuclear (Boston, MA, USA). Plasma immunoreactive insulin (IRI) was assayed by the radioimmunoassay using dextran-coated charcoal (Sasaki and Takahashi 1980).

Statistics

The mean values and the standard errors of the means were calculated. In the first experiment, the significant differences between saline and clonidine infusions were analyzed by analysis of variance using the General Linear Model (GLM) of the SAS program package (SAS Institute, Cary, NC, USA) on values pooled during infusions over time. In the second experiment, the data were assigned to six groups as represented in Tables. Significant differences between doses of drugs within each group were tested by analysis of variance using the GLM on values pooled within each group over time, and followed by Duncan's multiple range test.

RESULTS

Effect of clonidine on insulin and glucagon secretion (Fig. 1)

The plasma levels of IRI, IRG and glucose were unchanged during saline infusion. By contrast, the clonidine infusion brought about a slight decrease in plasma IRI from the pre-infusion level of $8.3 \pm 0.7 \mu\text{U/ml}$ to the average value during clonidine infusion of $2.8 \pm 0.1 \mu\text{U/ml}$. There was small but significant difference in plasma IRI between clonidine and saline infusions ($p < 0.05$).

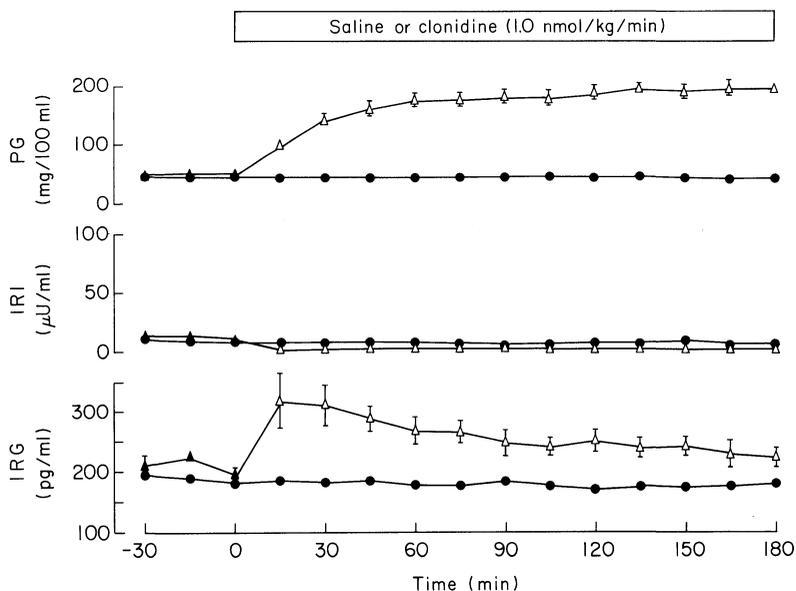


Fig. 1. Effect of clonidine infusion on plasma glucose, insulin and glucagon concentrations. Four sheep each received intravenous infusion of saline (●) or clonidine (1.0 nmol/kg/min, ▲) for 180 min. Mean values for 4 sheep with their standard errors are shown by vertical bars. Significant differences between saline and clonidine infusions were analyzed by analysis of variance using the GLM on values pooled during infusions over time. Open symbols (△) indicate significant differences ($p < 0.05$) compared with saline infusion (●).

Plasma IRG increased after the clonidine infusion from the baseline of 195 ± 12 pg/ml to 320 ± 47 pg/ml at 15 min, and decreased gradually but maintained high level throughout the clonidine infusion. There was statistical difference in plasma IRG during the clonidine infusion compared with the saline infusion ($p < 0.05$). Clonidine produced a marked increase in plasma glucose concentration from the baseline of 50.6 ± 3.2 mg/100 ml to a peak value of 197 ± 12.8 mg/100 ml at 165 min and hyperglycemia was maintained throughout the experimental period ($p < 0.05$).

Effects of midaglizole and yohimbine on insulin and glucagon secretion during clonidine infusion

As shown in Fig. 2, plasma IRI decreased during the concomitant infusion of clonidine and saline from the pre-infusion value of 11.5 ± 1.2 to 1.6 ± 0.4 μ U/ml at 40 min. The concomitant infusion of midaglizole at the highest dosage of 50 nmol/kg/min during the clonidine infusion, midaglizole did not cause any significant changes in plasma IRI (Fig. 2, Table 1). Whereas the concomitant yohimbine infusion produced a marked increase in plasma IRI from 5.0 ± 1.5 μ U/ml at 120 min to 114.8 ± 65.1 μ U/ml at 150 min, which was followed by a rapid

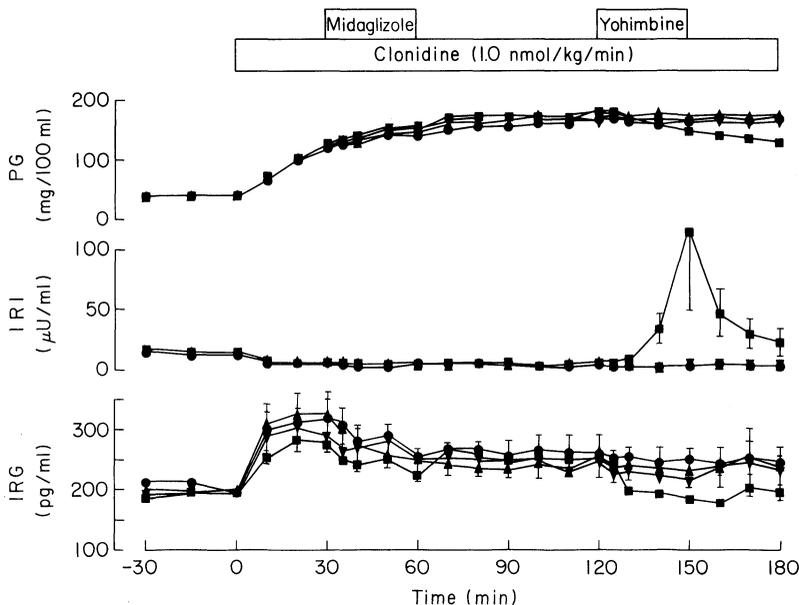


Fig. 2. Effects of α_2 -adrenergic antagonists (midaglizole and yohimbine) infusions on plasma insulin, glucagon and glucose concentrations during clonidine infusion (1.0 nmol/kg/min). α_2 -Adrenergic antagonists were infused at concentrations of 0 (\bullet), 5 (\blacktriangle), 10 (\blacktriangledown) and 50 nmol/kg/min (\blacksquare). Mean values for 4 sheep with their standard errors are shown by vertical bars. The results of statistical analysis are represented in Tables.

TABLE 1. *The effects of midaglizole and yohimbine on clonidine-induced IRI responses ($\mu\text{U}/\text{ml}$)*

| Dosage of α_2 -antagonist (nmol/kg/min) | -30~0 | 10~30 | 35~60 | 90~120 | 125~150 | 160~180 (min) |
|--|-------------------|------------------|------------------|------------------|---------------------------------|---------------------------------|
| Saline | 12.3 ± 2.1 | 4.0 ± 1.7 | 2.4 ± 1.0 | 2.5 ± 1.0 | 2.0 ^a ± 0.4 | 2.4 ^a ± 0.8 |
| 5.0 | 14.5 ± 3.5 | 5.8 ± 2.2 | 4.7 ± 1.7 | 3.2 ± 1.3 | 3.0 ^a ± 1.2 | 3.7 ^a ± 1.6 |
| 10.0 | 11.4 ± 0.9 | 3.0 ± 0.9 | 2.6 ± 1.0 | 2.2 ± 0.8 | 3.5 ^a ± 0.8 | 4.9 ^a ± 1.7 |
| 50.0 | 12.6 ± 1.8 | 3.8 ± 1.5 | 2.9 ± 0.6 | 3.1 ± 0.9 | 40.4 ^b ± 23.4 | 32.6 ^b ± 16.0 |
| <i>p</i> -Value | 0.4106 | 0.2134 | 0.0524 | 0.5890 | 0.0260 | 0.0001 |

Values represent means \pm s.e. for 4 sheep.

p-Value, significant differences between doses of drugs within each group were tested by analysis of variance using the GLM on values pooled within each group over time.

^{a,b}means with no common superscripts are significantly different ($p < 0.05$) by Duncan's multiple range test.

Clonidine was infused for 180 min from 0 to 180 min at a dose of 1.0 nmol/kg/min, Midaglizole was infused for 30 min from 30 to 60 min, Yohimbine was infused for 30 min from 120 to 150 min.

TABLE 2. *The effects of midaglizole and yohimbine on clonidine-induced IRG responses (pg/ml)*

| Dosage of α_2 -antagonist (nmol/kg/min) | -30~0 | 10~30 | 35~60 | 90~120 | 125~150 | 160~180 (min) |
|--|---------------------|---------------------|---------------------|---------------------|----------------------------------|---------------------|
| Saline | 204.6 ± 9.7 | 308.0 ± 32.7 | 281.3 ± 21.6 | 260.1 ± 33.4 | 249.0 ^a ± 18.3 | 245.3 ± 17.1 |
| 5.0 | 197.2 ± 5.4 | 319.3 ± 37.8 | 269.4 ± 28.6 | 239.9 ± 24.2 | 235.3 ^a ± 24.4 | 243.1 ± 44.5 |
| 10.0 | 193.3 ± 14.2 | 292.1 ± 36.9 | 265.3 ± 18.0 | 241.1 ± 12.5 | 221.8 ^a ± 15.3 | 237.8 ± 31.7 |
| 50.0 | 192.6 ± 7.2 | 270.8 ± 13.3 | 242.1 ± 12.4 | 247.5 ± 12.6 | 202.6 ^b ± 6.6 | 192.2 ± 11.5 |
| <i>p</i> -Value | 0.4429 | 0.2518 | 0.0845 | 0.5403 | 0.0033 | 0.0737 |

Values represent means \pm s.e. for 4 sheep.

Statistics were explained in Table 1.

^{a,b}means with no common superscripts are significantly different ($p < 0.05$) by Duncan's multiple range test.

Clonidine was infused for 180 min from 0 to 180 min at a dose of 1.0 nmol/kg/min, Midaglizole was infused for 30 min from 30 to 60 min, Yohimbine was infused for 30 min from 120 to 150 min.

TABLE 3. *The effects of midaglizole and yohimbine on clonidine-induced hyperglycemia (mg/100 ml)*

| Dosage of α_2 -antagonist (nmol/kg/min) | -30~0 | 10~30 | 35~60 | 90~120 | 125~150 | 160~180 (min) |
|--|--------------|--------------|----------------|----------------|----------------|-----------------------------|
| Saline | 38.4 ±0.9 | 94.4 ±7.4 | 135.7 ±10.1 | 161.1 ±10.0 | 163.2 ±8.2 | 168.1 ^a ±5.8 |
| 5.0 | 39.9 ±2.2 | 95.8 ±4.3 | 135.7 ±5.7 | 170.5 ±4.7 | 173.7 ±10.2 | 172.5 ^a ±10.7 |
| 10.0 | 37.2 ±1.3 | 97.4 ±6.3 | 143.9 ±4.5 | 164.3 ±11.3 | 165.9 ±8.5 | 161.4 ^a ±1.8 |
| 50.0 | 39.1 ±2.5 | 99.4 ±5.3 | 140.3 ±12.8 | 169.7 ±22.6 | 160.7 ±21.0 | 136.2 ^b ±16.9 |
| <i>p</i> -Value | 0.4234 | 0.9721 | 0.5145 | 0.6924 | 0.4830 | 0.0003 |

Values represent means ± s.e. for 4 sheep.

Statistics were explained in Table 1.

^{a,b}means with no common superscripts are significantly different ($p < 0.05$) by Duncan's multiple range test.

Clonidine was infused for 180 min from 0 to 180 min at a dose of 1.0 nmol/kg/min, Midaglizole was infused for 30 min from 30 to 60 min, Yohimbine was infused for 30 min from 120 to 150 min.

decrease after discontinuing yohimbine infusion (Fig. 2). The statistical differences in plasma IRI were observed at the highest dose of yohimbine ($p < 0.05$, Table 1).

The clonidine infusion at a rate of 1.0 nmol/kg/min caused an increase of plasma IRG from the initial level of 194 ± 9.7 to 316 ± 30.9 pg/ml at 30 min (Fig. 2). When the midaglizole was infused at the highest dosage of 50 nmol/kg/min, plasma IRG was decreased from the pre-infusion level of 277 ± 15.6 to 227 ± 10.4 pg/ml at 60 min. The greater the dose of midaglizole used, the less was the hyperglucagonemic response to clonidine, but this trend did not reveal any statistical difference (Table 2). On the other hand, the highest yohimbine infusion at a rate of 50 nmol/kg/min during the clonidine infusion produced a decrease of plasma IRG from 249 ± 13.3 pg/ml at 120 min to 178 ± 5.3 pg/ml at 160 min. Furthermore, as shown in Table 2, a significant difference was observed in IRG responses between the highest dose of yohimbine and the other lower doses ($p < 0.01$).

A massive hyperglycemia induced by the clonidine infusion was not altered by the concomitant infusion of midaglizole or yohimbine (Fig. 2). The infusion of an α_2 -adrenergic antagonist, midaglizole, failed to get statistical difference of plasma glucose concentrations between the doses of midaglizole. By contrast, the highest dose of yohimbine infusion produced a decrease of the plasma glucose concentration from the pre-infusion level of 174.7 ± 21.8 mg/100 ml at 120 min to 129.6 ± 12.4 mg/100 ml at 180 min. The statistical significance was observed in

plasma glucose concentrations at the highest dose of yohimbine after discontinuing the yohimbine infusion ($p < 0.05$, Table 3).

DISCUSSION

The present results show that clonidine infusion caused hyperglucagonemia, hypoinsulinemia and hyperglycemia in conscious sheep, and that these phenomena were clearly counteracted by yohimbine infusion. These results are in good agreement with our previous findings (Oda et al. 1990) that an α_2 -adrenergic mechanism was more important for the stimulation of pancreatic glucagon secretion and the inhibition of insulin secretion than an α_1 -adrenergic mechanism in sheep.

It is well known that the insulin secretion was enhanced by β -adrenergic stimulation and inhibited by α -adrenergic activation in human (Porte 1967), in dogs (Iversen 1973; Samols and Weir 1979), in rats (Nakhoda et al. 1981), in rabbits (Knudtzon 1984a), in calves (Bloom and Edwards 1978), in sheep (Sasaki et al. 1982; Oda et al. 1988) and in goats (Oda et al. 1986). Therefore, as far as insulin secretion is concerned, the effect of adrenergic stimulation is always consistent in various animal species.

There are, however, conflicting results concerning the adrenergic modulation of pancreatic glucagon secretion as to whether the secretion was activated through an α -adrenergic mechanism in dogs (Baum et al. 1979), in rabbits (Knudtzon 1984a), in calves (Bloom and Edwards 1978, 1984), in goats (Oda et al. 1986) and in sheep (Sasaki et al. 1982; Oda et al. 1988), or a β -adrenergic mechanism in human (Atkinson et al. 1981), in dogs (Iversen 1973; Boden et al. 1982) and in rats (Narimiya et al. 1981; Itoh and Gerich 1982) and or both α - and β -adrenergic mechanisms in rats (Nakhoda et al. 1981) and in dogs (Samols and Weir 1979).

Furthermore, there are also conflicting results about adrenergic subtype receptor modulation of pancreatic glucagon secretion. It has been demonstrated that yohimbine inhibited the glucagon response to epinephrine infusion in rats (Patel 1984), in rabbits (Knudtzon 1984b) and in sheep (Oda et al. 1990), but prazosin did not affect the epinephrine-induced glucagon secretion in rabbits (Knudtzon 1984b) and in sheep (Oda et al. 1990). In addition, xylazine, an α_2 -adrenergic agonist, also induced hyperglucagonemia in sheep (Muggaberg and Brockman 1982). Furthermore, both phenylephrine and clonidine enhanced plasma glucagon levels in mouse (Skoglund et al. 1987). On the other hand, it has been shown that clonidine was ineffective on the glucagon secretion in human (Ferlito et al. 1985), in rat islet cells (Schuit and Pipeleers 1986) and in rat pancreas (Filipponi et al. 1986). It has also been reported that the infusion of a highly selective β_1 -adrenergic antagonist, metoprolol, inhibited epinephrine-induced hyperglucagonemia (Gregorio et al. 1986). Thus, the adrenergic modulation of pancreatic glucagon secretion is not yet clear. However, the present experiments indicate that glucagon secretion is strongly affected by α_2 -adrenergic

receptors in sheep.

Our present results show that the clonidine infusion caused massive hyperglycemia, which was slightly attenuated by the yohimbine infusion. These results are also in good agreement with our previous findings that epinephrine-induced hyperglycemia was eliminated by yohimbine, but not by prazosin infusion in sheep (Oda et al. 1990), and with the findings in rabbits (Knudtson 1984b). Hyperglycemia induced through the clonidine infusion was derived from direct effect of clonidine upon liver glycogenolysis (Exton 1980) as well as the secondary effect of decreased insulin secretion and increased glucagon secretion.

Midaglizole is a selective α_2 -adrenergic antagonist (Kameda et al. 1982; Muramatsu et al. 1983). Midaglizole administration stimulated insulin secretion and inhibited blood glucose level in rats (Kameda et al. 1982; Koh et al. 1987). It has been proposed that the enhanced insulin secretion was induced by the release from the adrenergic inhibition (Koh et al. 1987). Moreover, it has also been reported that midaglizole has lower affinity for α_2 -adrenergic receptors than yohimbine in the dog mesenteric artery and the rat vas deferents (Muramatsu et al. 1983). In the present experiments, midaglizole showed a tendency as an α_2 -adrenergic antagonist as observed in the changes in plasma IRG but the action was clearly less than that of yohimbine (Fig. 2).

It is concluded from the present experiments that the glucagon secretion was stimulated and the insulin secretion was inhibited by the α_2 -adrenergic subtype mechanism in conscious sheep. The α_2 -adrenergic antagonistic effect of midaglizole was weaker than that of yohimbine in sheep.

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