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ORIGINAL ARTICLE

Effects of dexmedetomidine on insulin secretion from rat pancreatic β cells

Tetsuya Takahashi · Takashi Kawano · Satoru Eguchi · Haidong Chi · Hideki Iwata · Masataka Yokoyama

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Abstract
Purpose Dexmedetomidine acts as a selective α_2-adrenergic receptor agonist and an imidazoline receptor agonist, both of which are known to affect insulin secretion. Here, we investigated the effects of clinically relevant concentrations of dexmedetomidine on insulin secretion under in vivo conditions. Furthermore, its underlying mechanisms were examined using isolated islets in vitro.
Methods For the in vivo oral glucose tolerance test (OGTT), male Sprague–Dawley rats were randomly allocated to one of three groups ($n = 7$ in each group): two groups infused with dexmedetomidine at a low (group L) or a high (group H) dose, and one control group infused with the same amount of saline (group C). For the in vitro perifusion study, insulin released from isolated islets was measured during stepwise changes in glucose. Dexmedetomidine (0.1–100 μM) was added to the chamber.
Results During the OGTT test, the insulin levels in group H were significantly lower than those in group C at 30, 60, and 90 min after glucose load. On the other hand, insulin levels in group L were comparable to those of group C at all time points. In the perfusion study, dexmedetomidine inhibited glucose-stimulated insulin secretion in a concentration-dependent manner. When co-treated with yohimbine, an α_2-adrenoceptor blocker, dexmedetomidine adversely increased glucose-induced insulin secretion. However, co-treatment with idazoxan, an antagonist for α_2-adrenergic and imidazoline receptors, completely abolished the action of dexmedetomidine.
Conclusions Dexmedetomidine had no effect on insulin secretion at sedative dose, whereas it significantly inhibited insulin secretion at supraclinical high concentrations mainly via the α_2-adrenoceptor.

Keywords Dexmedetomidine · Insulin · α_2-Adrenoceptor · Imidazoline

T. Takahashi · T. Kawano (✉) · H. Chi · H. Iwata · M. Yokoyama
Department of Anesthesiology and Intensive Care Medicine, Kochi Medical School, Kohasu, Oko-cho, Nankoku, Kochi 783-8505, Japan
e-mail: takashika@kochi-u.ac.jp

S. Eguchi
Department of Dental Anesthesiology, Tokushima University School of Dentistry, Tokushima, Japan

Introduction

Hyperglycemia during the perioperative period is known to be associated with the risk of relevant postoperative outcomes such as infectious complications [1–3]. Surgical stress causes a stereotypical metabolic response, including increased circulating levels of catecholamines, cortisol, glucagon, and growth hormones, which inhibit insulin secretion and induce insulin resistance [1–3]. Furthermore, several general anesthetics, including sevoflurane, isoflurane, and propofol, have been reported to affect glucose metabolism, as well as insulin secretion [4–7].

It is well established that activation of α_2-adrenergic receptors inhibits insulin secretion [8, 9]. Dexmedetomidine, an α_2-adrenoceptor agonist, has been widely used in clinical practice as an anesthetic adjunct in the perioperative period [10]. In addition, dexmedetomidine has an imidazole ring in its structure and interacts with imidazoline receptors [11]. Some imidazoline derivatives have been shown to increase the insulin response to glucose [12]. These pharmacological properties of dexmedetomidine may contribute significantly to modulate insulin secretion and glucose tolerance via either α_2-adrenergic signaling

and/or imidazoline receptors. However, little is known about the effects of dexmedetomidine on insulin secretion. In the present study, we investigated whether dexmedetomidine at a sedative dose can affect insulin secretion using rat in vivo mode. Furthermore, its underlying mechanisms were explored by in vitro antagonistic experiment.

Materials and methods

The study protocol was approved by the Kochi Medical School Animal Care and Use Committee. The experimental animals were male Sprague–Dawley rats aged 9 weeks and weighing 300–350 g. They were housed individually in a temperature-controlled environment (22 ± 0.5 °C) with a 12-h light/dark cycle, and provided with food and water ad libitum until 6 h before the experiments.

General preparation

The general animal preparation for in vivo experiment was the same as described previously, with modification [13]. Briefly, all rats were subjected to laparotomy under isoflurane anesthesia (1.5–3 % in oxygen), and a second silicone catheter was advanced into the stomach via a small enterotomy made in the anterior wall of the stomach as a gavage tube. Animals were further implanted with an indwelling vascular catheter in the right femoral vein and left femoral artery to allow intravenous injections and arterial blood sampling, respectively. Body temperature was measured with a rectal probe and maintained at 37 °C with a heating pad during anesthesia. Each day, the catheters were infused with 0.1 ml of a sterile saline solution containing heparin (30.0 IU/ml) to prevent the formation of clots. After surgery, rats were allowed to recover for a week; only the rats that regained their pre-treatment body weight were subjected to further examination.

Oral glucose tolerance test

Rats were randomly allocated to one of three groups ($n = 7$ per group): two groups infused with dexmedetomidine (Sigma-Aldrich, St. Louis, MO, USA) at a low (group L; 0.5 μg/kg for bolus injection, and then at 3.6 μg/kg/h for continuous infusion) or high (group H; 1.5 μg/kg for bolus injection, and then at 10.8 μg/kg/h for continuous infusion) dose and one control group infused with the same amount of saline (group C). The infusion was continued until the end of the experiments via the femoral vein by an infusion pump (ATOM 1235; Nipro, Japan). Low and high dose of dexmedetomidine were determined on the basis of a previous study [14], corresponding to steady-state plasma levels (achieved within 5–10 min) of approximately 0.5 and 1.5 ng/ml, respectively. Mean arterial blood pressure (MAP) and heart rate (HR) were measured noninvasively using the rat tail cuff method during experiments. Arterial blood (0.3 ml) was drawn from the left femoral artery before the beginning of infusion (baseline) and at the end of infusion to determine blood gas tensions.

Fifteen minutes after initiation of infusion, an oral glucose tolerance test (OGTT) was performed using an oral 2.0 g/kg glucose (using 500 mg glucose/ml distilled H_2O) challenge. The oral glucose load was given as gavage via a gastric tube connected to a syringe to ensure accurate dosing. Blood samples (100 μl/time point) were collected from the tail vein in centrifuge tubes at −30 min (before starting infusion), time 0 (prior to the glucose load), and 30, 60, 90, and 120 min after the glucose load. Serum glucose and insulin concentrations were measured using the glucose oxidase method (Tido-Tidex; Sankyo, Tokyo, Japan) and a commercially available insulin enzyme-linked immunosorbent assay (Morinaga Institute of Biological Science, Yokohama, Japan), respectively. The grade of sedation was evaluated at baseline and time 0 and 120 min by observations of behavioral and reflex activity according to a method reported previously [1].

Pancreatic islet and cell preparation

Rats were lightly anesthetized with ether and killed by cervical dislocation, and pancreatic islets were isolated by collagenase (Type IV, Sigma-Aldrich, St. Louis, MO, USA) digestion as described previously [13]. To ensure 100 % purity of the preparation, islets were purified by discontinuous density gradients, hand-picked, and counted under a stereomicroscope. Isolated islets were cultured overnight in RPMI 1640 medium (Sigma-Aldrich) containing 10 % fetal bovine serum (Sigma-Aldrich), 100 U/ml penicillin, 100 μg/ml streptomycin, and 11 mM glucose to allow recovery from the isolation procedure.

Perifusion study

A perifusion chamber was placed in an incubator, and the experiments were conducted at 37 °C in an atmosphere of 95 % air and 5 % CO_2. Twenty isolated islets (150–200 μm in diameter) were placed in the perifusion chamber and perifused at a rate of 1 ml/min with Krebs–Ringer buffer. Five separate experiments were performed using different preparations of rat pancreatic islets for a total period of 40 min. Following at least a 30-min equilibration period, the glucose concentration was increased experimentally from 2.8 mM (10 min; 0–10 min) to 16.7 mM (24 min; 10–34 min) and then returned to 2.8 mM (6 min; 34–40 min). Dexmedetomidine (0.1–10 μM) or its vehicle (saline) was added to the chamber from 3 to 34 min. The effluents from

Table 1 Physiologic variables during oral glucose tolerance tests

	Baseline	Before glucose	After glucose		
			30 min	60 min	120 min
HR (min^{-1})					
Group C	374 ± 23	371 ± 33	377 ± 29	378 ± 24	376 ± 28
Group L	379 ± 29	329 ± 27	321 ± 21	322 ± 16	326 ± 28
Group H	376 ± 18	310 ± 16*	313 ± 23*	303 ± 28*	311 ± 29*
MAP (mmHg)					
Group C	108 ± 7	108 ± 8	107 ± 8	107 ± 6	109 ± 7
Group L	105 ± 9	106 ± 7	103 ± 4	104 ± 6	104 ± 6
Group H	109 ± 4	104 ± 2	104 ± 4	106 ± 7	102 ± 9
$PaCO_2$ (mmHg)					
Group C	35.7 ± 2.3	–	–	–	36.2 ± 2.8
Group L	34.8 ± 2.8	–	–	–	35.7 ± 2.4
Group H	36.2 ± 3.9	–	–	–	37.7 ± 3.9
PaO_2 (mmHg)					
Group C	92.8 ± 3.6	–	–	–	93.5 ± 4.2
Group L	90.1 ± 4.4	–	–	–	91.2 ± 4.8
Group H	91.4 ± 5.1	–	–	–	92.5 ± 3.4

MAP mean arterial blood pressure, *HR* heart rate, $PaCO_2$ partial pressure of carbon dioxide, PaO_2 partial pressure of oxygen

* $p < 0.05$, compared with baseline within group

the perifusion chamber were collected every minute and frozen for insulin determination by insulin ELISA assay as described above.

Drugs

The following drugs were used in the study: dexmedetomidine (Hospira Japan Co., Osaka, Japan), yohimbine (Sigma-Aldrich), and idazoxan (Sigma-Aldrich). All compounds were dissolved in 0.9 % physiological saline.

Statistics

All data have been presented as mean ± SD. Differences between the data sets were evaluated by performing repeated-measures one-way analysis of variance, followed by Bonferroni post hoc tests. Student's *t* tests were also used for pair-wise comparisons whenever required. Non-interval data were analyzed by using the Kruskal–Wallis test. A *p* value of 0.05 was considered statistically significant. The area under the curve was determined using curve analysis software (GraphPad, Prism, San Diego, CA, USA).

Results

Physiologic measurements during OGTT

There were no significant differences in all measurements between groups under baseline conditions (Table 1). Fifteen minutes after initiating dexmedetomidine infusion, HR decreased in group L and group H compared with baseline or group C, and remained steady until the end of the experiments. On the other hand, MAP, $PaCO_2$, and PaO_2 were not different at any time points within or between groups.

The levels of sedation recorded at 0 and 120 min (15 and 135 min after dexmedetomidine infusion, respectively) are shown in Fig. 1. Dexmedetomidine infusion resulted in a dose-dependent sedation. The highest grade of sedation, 2, represents somnolence characterized by loss of righting reflex while the tail pinch response is still intact, and was achieved in all rats of group H. Kruskal–Wallis analysis demonstrated significant differences in sedation between groups, but comparable within groups at 0 and 120 min.

Glucose and insulin levels during OGTT

We compared OGTT indices between the three groups to clarify the effects of dexmedetomidine sedation on acute glucose homeostasis and insulin release in vivo. The mean glucose and insulin responses during OGTT are shown in Fig. 2a, b respectively. Fasting concentrations of insulin and glucose measured before administration of dexmedetomidine (−30 min) and just before administration of glucose (0 min) were comparable across groups. After administration of 2.0 g/kg glucose, blood glucose concentrations increased and then returned to baseline levels within 2 h in all experimental groups. Rats in group H showed delayed glucose clearance with glucose levels greater than those in group C at 30 and 60 min after glucose load. The insulin

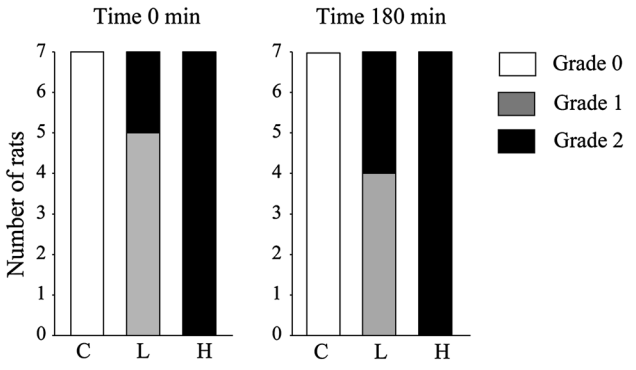

Fig. 1 Distribution of sedation grades in rats infused with control saline (*C*), low-dose dexmedetomidine (*L*), or high-dose dexmedetomidine (*H*). Grades were assessed at time 0 and 180 min on a three-point scale according to Tanaka et al. [13], where *0* = resting quietly, intermittent spontaneous activity, *1* = sedated and arousable with difficulty with diminished whisker reflex, but with intact righting reflex, and *2* = never arousable, with diminished righting reflex. The *columns* indicate the number of rats out of total rats ($n = 7$) in each group

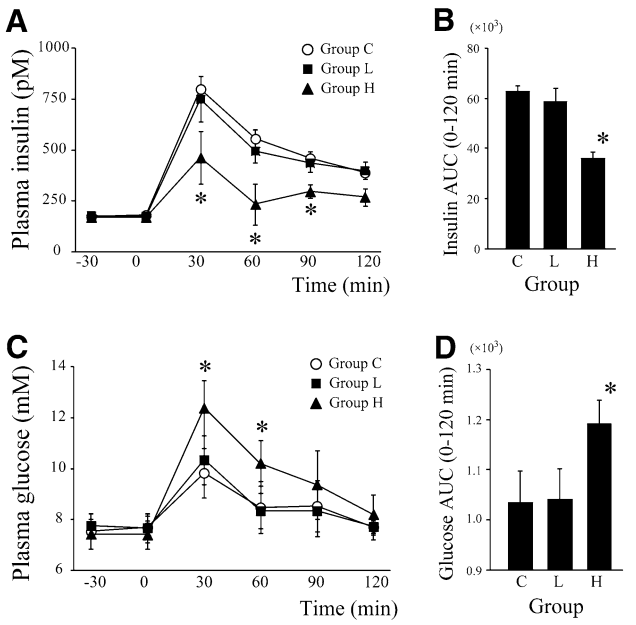

Fig. 2 Blood plasma insulin (**a**, **b**) and glucose (**c**, **d**) levels during an oral glucose tolerance test for rats infused with control saline (group C), low-dose dexmedetomidine (group L), or high-dose dexmedetomidine (group H). **b** and **d** were obtained by taking the area under the curve (AUC) from the time course studies in **a** and **c**, respectively. Values are expressed as mean ± SD. *$p < 0.05$ vs. group *C*

levels in group H were lower than those in group C at 30, 60, and 90 min after glucose load. On the other hand, both glucose and insulin levels in group L were comparable to those of group C at all time points. The total glucose area under the curve (glucose AUC) significantly increased in

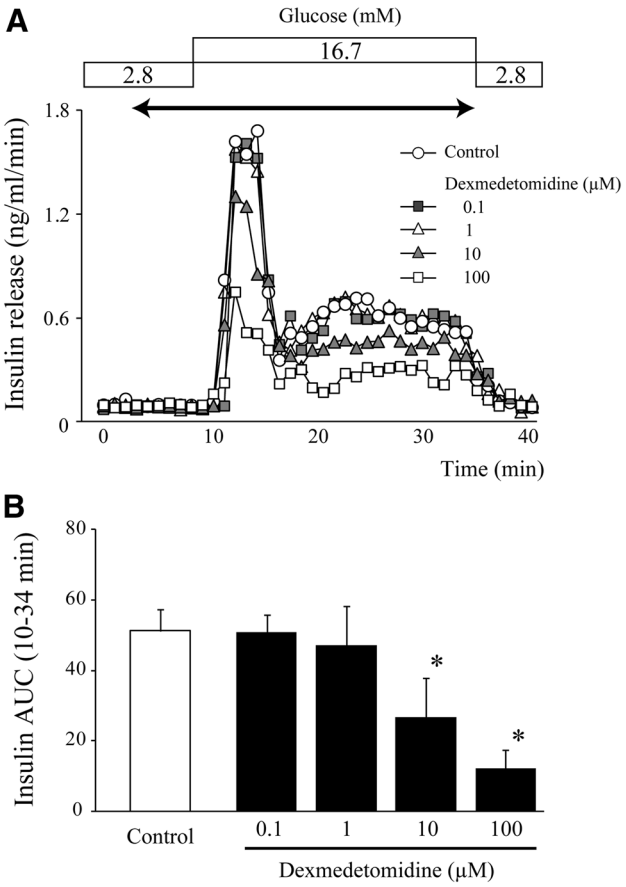

Fig. 3 Effects of dexmedetomidine (0.1–100 μM) on glucose-stimulated insulin release in pancreatic islets isolated from rats. **a** Dexmedetomidine (or vehicle control) was added to the chamber from 3 to 34 min indicated by *horizontal double arrow*. Each *symbol* represents the mean value derived from five independent experiments in each concentration tested. **b** Total glucose-stimulated insulin secretion, calculated by integrating area under curve (AUC), during the 16 mM glucose challenge period (10–34 min). Data are presented as mean ± SD. *$p < 0.05$ vs. control

group H, but not in group L, compared with group C. The total insulin area under the curve (insulin AUC) decreased in group H, but not group L, compared with group C.

Insulin secretion from isolated rat islets

To confirm the differential effects of dexmedetomidine on glucose-induced insulin secretion in vitro, we measured insulin release from an isolated rat islet perfusion system during stepwise changes in glucose concentrations from 2.8 to 16.7 mM. As shown in Fig. 3a (control), superfusion with 16.7 mM glucose induced a biphasic insulin secretory response. However, bath application with dexmedetomidine (0.1, 1, 10, 100 μM) inhibited both phases of glucose-stimulated insulin secretion in a concentration-dependent manner. The relationship between relative insulin AUC and

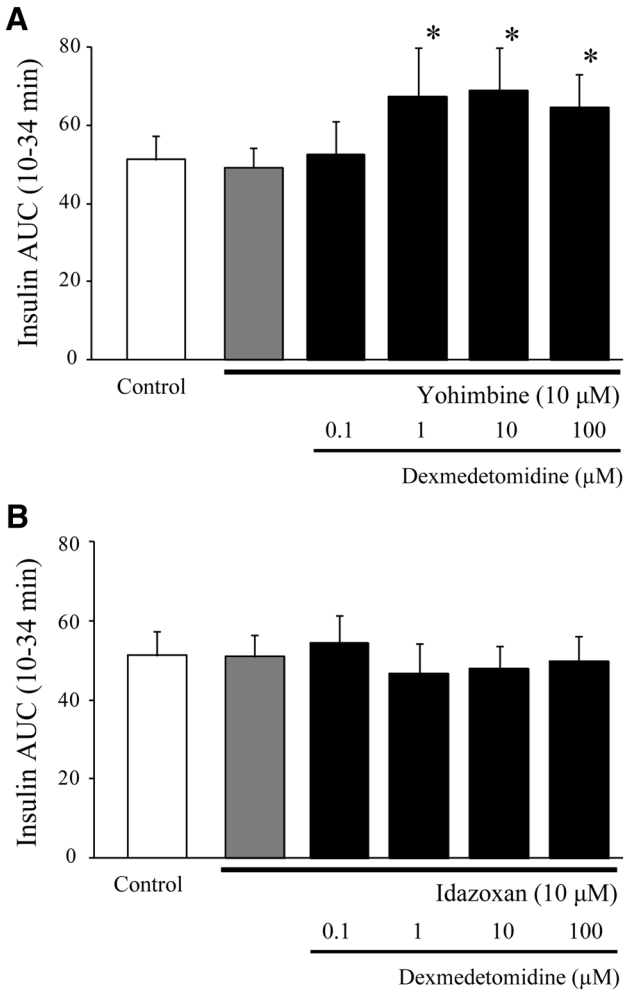

Fig. 4 Effects of concomitant treatment with yohimbine (**a**) or idazoxan (**b**) on dexmedetomidine-induced dose-dependent inhibition of glucose-stimulated insulin release from pancreatic islets. Total glucose-stimulated insulin secretion, calculated by integrating area under curve (AUC), during the 16 mM glucose challenge period (10–34 min). Data are presented as mean ± SD. *$p < 0.05$ vs. control

concentrations of dexmedetomidine is shown in Fig. 3b. Compared with vehicle control, dexmedetomidine significantly inhibited glucose-stimulated insulin secretion at concentrations of 10 μM or greater.

Involvement of α_2-adrenoceptors and imidazole binding sites

Dexmedetomidine is known to have high binding affinity for both α_2-adrenergic and imidazole receptors. To test whether these receptors were involved in the dexmedetomidine-induced inhibition of insulin secretion from isolated rat islets, the effects of a selective α_2-adrenoceptor blocker, yohimbine, and a dual antagonist for α_2-adrenergic and imidazoline receptors, idazoxan, were examined.

When co-treated with yohimbine (10 μM), dexmedetomidine adversely increased glucose-induced insulin secretion (Fig. 4a). These insulinotropic effects were observed in both first- and second-phase insulin secretion (data not shown). On the other hand, co-treatment with idazoxan (10 μM), an antagonist for α_2-adrenergic and imidazoline receptors, completely abolished the action of dexmedetomidine (Fig. 4b). Neither yohimbine nor idazoxan alone had effects on insulin release from isolated islets (data not shown).

Discussion

In the present study, we showed that dexmedetomidine dose-dependently inhibits glucose-induced insulin release in both an in vivo OGTT and an in vitro isolated islet perifusion study. The antagonist experiments further demonstrated that dexmedetomidine has dual action on insulin secretion: predominant inhibition via the α_2-adrenoceptor and weak stimulation via the imidazoline receptor.

It has been reported that the stimulation of α_2-adrenoceptor on pancreatic β cells an inhibition of insulin secretion [8, 9]. On the other hand, clinically relevant doses of dexmedetomidine are widely known to induce sedative and analgesic effects mediated by the stimulation of α_2-adrenoceptors of the central nervous system [10]. However, higher doses of dexmedetomidine can stimulate the peripheral α_2-adrenoceptors in addition to the effects on the central α_2-adrenoceptors [10]. Therefore, the dexmedetomidine-induced inhibition of insulin secretion observed in the present experiments may have resulted by the activation of peripheral (pancreatic) α_2-adrenoceptors.

In the current in vivo experimental settings, the rats that received low-dose dexmedetomidine showed a light sedative condition (Fig. 2), with preserved righting reflex, which precisely mimics the clinical situation in humans. However, in this low-dose group, dexmedetomidine-induced inhibition of insulin release was not observed (Fig. 2a, b). In contrast, in the rats that received high-dose dexmedetomidine, the inhibition of insulin release occurred. These rats showed a deep sedative condition, which may be above the clinically recommended doses. These findings imply that dexmedetomidine can affect insulin secretion only if used over the sedative dosages.

In the in vitro superfusion study, dexmedetomidine inhibited glucose-induced insulin secretion at concentrations above 10 μM. The therapeutic plasma concentration range of dexmedetomidine is reported to be 0.27–1.37 ng/ml (approximately 1.0–6.0 nM) [15]. Thus, the concentrations of dexmedetomidine that significantly inhibited insulin secretion in the current study are very high, suggesting that dexmedetomidine-induced inhibition of

glucose-induced insulin secretion was observed at concentrations above clinically recommended values. Meanwhile, recently, contradictory results have also been reported. Kodera et al. [16] reported that dexmedetomidine at 1 pM or more dose-dependently inhibited the insulin secretion elicited by 8.3 mM glucose in islet perfusion experiments. This discrepancy in results could at least partly be explained by difference in islet culture conditions; i.e., overnight incubated in our study vs. fleshly isolated in Kodera's study. Nevertheless, since dexmedetomidine has high affinity to α_2-adrenoceptors (pEC_{50}/pKi with 8–9; nM order) [17], we cannot rule out the possibility that dexmedetomidine could modulate insulin secretion at lower but clinically relevant concentrations.

Dexmedetomidine pharmacologically acts as an agonist for α_2-adrenoceptors as well as for imidazoline receptors [11]. Furthermore, similar to the α_2-adrenoceptors, the imidazoline receptors are also expressed in pancreatic β cells, and their activation could cause an increase in insulin release via the inhibition of ATP-sensitive potassium channel or some other unknown mechanism [12]. In the present study, pretreatment with yohimbine, a selective antagonist for α_2-adrenoceptors, did not diminish the dexmedetomidine-induced inhibition of insulin release, but enhanced the insulin release (Fig. 4a). These results suggest that α_2-adrenoceptors are antagonized by yohimbine, which unmasked the effects of dexmedetomidine as an imidazoline agonist. Indeed, pretreatment with idazoxan, an antagonist for both α_2-adrenoceptors and imidazoline receptors, completely diminished the dexmedetomidine-induced inhibition of insulin release (Fig. 4b). On the other hand, dexmedetomidine-induced increase in insulin secretion observed after treatment with yohimbine was relatively small and showed no concentration dependency. Furthermore, the present study and a previous study [16] showed that this insulinotropic effect was not observed at low (clinically relevant) concentrations of dexmedetomidine (0.1 μM or less). Accordingly, dexmedetomidine-induced increase in insulin secretion via imidazoline receptors may be seen only at supraclinical concentrations, and minor compared to dexmedetomidine-induced inhibition of insulin secretion via α_2-adrenoceptors.

Hyperglycemia is an important independent risk factor associated with patient outcomes [1–3]. Especially in perioperative periods, hyperglycemia is prone to develop due to nutrient starvation and surgical invasion. In addition, volatile anesthetics have been reported to directly inhibit insulin secretion in pancreatic β cells [4, 5]. On the other hand, intravenous anesthetic propofol has no effect on insulin secretion [6]. The present study further demonstrates that dexmedetomidine may not impair insulin secretion if used at clinically relevant doses. However, higher doses of dexmedetomidine could be an influence in insulin secretion. Further research regarding the effects of dexmedetomidine on insulin release must be carried out in clinical settings.

In conclusion, dexmedetomidine inhibits insulin secretion from pancreatic β cells, mainly mediated by α_2-adrenoceptors. Since dexmedetomidine-induced inhibition of insulin secretion was found only at the above clinical concentrations, a common dose of dexmedetomidine for sedation may have no effect on insulin secretion.

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