Effects of 3-Hydroxybutyrate and Hyperosmolarity on Glucagon Release from Isolated Perfused Canine Pancreas

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Abstract

The effects of 3-hydroxybutyrate (3-OHB) and hyperosmolarity on glucagon secretion were examined in the isolated perfused canine pancreas. When 3-OHB was infused for 15 min into the pancreas perfused with 2.8 mM glucose, 5 and 20 mM sodium 3-OHB inhibited it after a transient stimulation, whereas a similar transient stimulation was observed also by the infusion of 20 mM NaCl in a control experiment. The above inhibition was not observed under the perfusate condition of 5.5 mM glucose plus 10 mM arginine. When the isolated canine pancreas was perfused under the perfusate condition of acidosis (pH 7.1), ketoacidosis (pH 7.1 and 20 mM 3-OHB) or hyperosmolarity (+60 mOsm/kg with sucrose) throughout the experiment, the glucagon concentrations produced by 2.8 mM glucose under the ketoacidotic and hyperosmolar conditions, were less than half of those obtained under the standard condition. The insulin level was not influenced by the above perfusate conditions. These results suggest that 3-OHB inhibits glucagon secretion stimulated by glucopenia, but does not inhibit it stimulated by amino acids, and that hyperosmolarity inhibits glucagon secretion but does not inhibit insulin secretion. The pathophysiological significance of these results must be slight, considering the presence of hyperglucagonemia during prolonged starvation or diabetic ketoacidosis,

Hyperglucagonemia during diabetic ketoacidosis and non-ketotic hyperosmolar coma in diabetic patients has been well established (Müller et al., 1973; Lindsey et al., 1974). The major cause of hyperglucagonemia is supposed to be augmented glucagon release provoked by hypovolemia and stress in addition to insulin deficiency and its decreased removal from the kidney (Unger and Orci, 1976). Although the increase in plasma 3-hydroxybutyrate (3-OHB) is mainly caused by enhanced ketone body production in the liver due to hyperglucagonemia, it has been reported that glucagon release is inhibited by 3-OHB (Edwards and Taylor, 1970). Namely, hyperketonemia and/or hyperosmolarity produced by hyperglucagonemia might directly influence the glucagon release from α-cells. However, reports concerning the
Effects of 3-OHB and osmolarity on glucagon secretion are scarce. We therefore examined the effects of 3-OHB and hyperosmolarity on glucagon release from the isolated perfused canine pancreas because 3-OHB mainly increases in the plasma during prolonged starvation and diabetic ketoacidosis.

Materials and Methods

Sodium DL-3-hydroxybutyrate was purchased from the Sigma Chemical Co. (St. Louis, Missouri, USA).

Pancreas perfusion: The pancreas was isolated from mongrel dogs weighing 14-17 kg under pentobarbital anesthesia after an overnight fast. The isolated canine pancreas was perfused by the method of Iversen and Miles (Iversen and Miles, 1971). A Krebs-Ringer bicarbonate buffer solution containing 4% dextran T-70 (Pharmacia Fine Chemicals, Upsala, Sweden), 0.2% BSA, 5 mM each of pyruvate, fumarate and glutamate (standard perfusate) was bubbled with a 95%O₂-5%CO₂ mixture at 37°C. The glucose and arginine concentrations in the perfusate are described in the figure legends. The flow rate of the perfusate was 16 ml/min and the venous perfusate was collected at 1 min intervals and stored at -40°C until the assay. In order to elicit the short term effects of 3-OHB, the experiment was performed with the 15 min-infusion of 3-OHB through a side-arm syringe at a rate of 0.2 ml/min after 55 min of pre-perfusion (Figure 1). In order to elicit the long term effects of acidosis (Figure 4), the pH was adjusted to 7.1 by reducing NaHCO₃ in the standard perfusate described above. The Na concentration was corrected with NaCl and the isolated pancreas was perfused with this perfusate from the beginning of perfusion. The ketoacidotic perfusate (20 mM 3-OHB and pH 7.1) was obtained by reducing NaHCO₃ in the standard perfusate and adding sodium 3-OHB. The long term effects of hyperosmolarity were observed with the perfusate containing 60 mM sucrose (+60 mOsm/kg) added to the standard perfusate.

Fig. 1. Changes in pancreatic hormone release from the isolated perfused canine pancreas in response to sodium 3-hydroxybutyrate (3-OHB). The basal perfusate contained 2.8 mM glucose or 5.5 mM glucose plus 10 mM arginine as shown in Figure. mean±SEM, A; N=4 and B; N=5. *, P<0.05 vs. mean of last four points during the control period.
perfusate because sucrose does not directly affect the pancreatic hormone release (Grill and Gerasi, 1976). After 55 min of perfusion with the above perfusates containing 2.8 mM glucose, the increase in the glucose and arginine concentrations was performed by the infusion of glucose and arginine through a side-arm syringe at a rate of 0.2 ml/min as shown in Figure 3. The response of α-cells and β-cells to glucose and arginine added under each perfusate condition was calculated as the area under the curve during each 15 min of infusion (Figure 4).

Hormone assay: Insulin, glucagon and somatostatin were assayed by RIAs, according to the methods of Herbert et al. (Herbert et al., 1965), Faloona and Unger (Faloona and Unger, 1974) and Harris et al. (Harris et al., 1978), respectively, as previously described (Chiba et al., 1985).

The ANOVA with Newman-Keuls procedure for multiple comparisons (Figure 1, 2) and the Student’s t-test for unpaired data were used (Figure 4) for statistical analysis. All data are expressed as mean ± SEM and values of P < 0.05 were considered to be significant.

Fig. 2. Effects of 20 mM NaCl on glucagon release from the isolated perfused canine pancreas under two perfusate conditions: 2.8 mM glucose and 5.5 mM glucose plus 10 mM arginine. Mean ± SEM, N = 5. *, P < 0.05 vs. mean of last four points during the control period.
Results

Effect of 3-OHB on glucagon release from the isolated perfused canine pancreas.

Figure 1 shows the effects of 3-OHB on pancreatic hormone release from the isolated perfused canine pancreas. Insulin and somatostatin releases were clearly stimulated by 5 and 20 mM sodium 3-OHB under the perfusate condition of 2.8 mM glucose. On the other hand, the glucagon release transiently increased immediately after the infusion of 3-OHB and decreased to less than its basal level within a few minutes.

Under the perfusate condition of 5.5 mM glucose plus 10 mM arginine, the increase in glucagon release was sluggish and the glucagon level did not decrease to less than the basal level during the infusion of 3-OHB. Insulin release was greatly stimulated by 20 mM 3-OHB and somatostatin release.
Fig. 4. Effects of acidosis (pH 7.1), ketoacidosis (20 mM 3-OHB, pH 7.1) and hyperosmolarity (+60 mOsm/kg) on glucagon (A) and insulin (B) release from the isolated perfused canine pancreas. The glucose and arginine were added to the perfusate as shown in Figure 3 and the cumulative glucagon and insulin outputs during each 15 min period were calculated as described in Materials and Methods. *, P<0.05 vs. control (perfusion with the standard perfusate described in Materials and Methods).

was stimulated as much as that observed under the perfusate condition of 2.8 mM glucose. Since Na⁺ directly affects pancreatic hormone secretion (Hales and Milner, 1968; Hermansen, 1980) 20 mM NaCl was infused instead of 3-OHB as a control experiment (Figure 2). Although a similar pattern of increase in glucagon release to that observed with 3-OHB was obtained with 20 mM NaCl, no significant decrease in glucagon was obtained under the perfusate condition with 2.8 mM glucose. During the perfusion with 5.5 mM glucose plus 10 mM arginine, a significant increase was also observed in the early phase after the infusion of 20 mM NaCl. 20 mM NaCl slightly stimulated the insulin release under the perfusate condition with 2.8 mM glucose, and a clear increase was observed with the condition of 5.5 mM glucose plus 10 mM arginine. Somatostatin release was slightly stimulated by 20 mM NaCl under the condition of 5.5 mM glucose plus 10 mM arginine.
Effects of acidosis, ketoacidosis and hyperosmolarity on glucagon response to glucose and arginine.

Figure 3 shows the change in glucagon and insulin levels in the effluent perfusate from the isolated canine pancreas in response to an increase in the glucose and arginine concentrations under the standard perfusate conditions described in Materials and Methods. The same protocol of perfusion was employed under the conditions of acidosis (pH 7.1), ketoacidosis (20 mM 3-OHB, pH 7.1) and hyperosmolarity (+60 mOsm/kg). The glucagon release during each 15 min period was calculated in each perfusate condition (Figure 4-A). Ketoacidosis suppressed the glucagon release induced by glucopenia (2.8 mM glucose). Hyperosmolarity suppressed glucagon release induced by both glucopenia (2.8 mM glucose) and arginine (22 mM glucose plus 10 mM arginine).

The insulin release was not inhibited significantly under these conditions (Figure 4-B). The insulin release suppressed by glucopenia was restored under the ketoacidotic condition.

Discussion

The present study demonstrates that 1) 3-OHB suppresses glucagon release stimulated by glucopenia (2.8 mM glucose) in both short term and long term experiments (Figure 1 and 4-A) and slightly augments it stimulated by arginine (Figure 1); 2) The rapid and transient increase in glucagon release brought about by sodium 3-OHB with 2.8 mM glucose is ascribed to the effect of Na⁺ in the sodium 3-OHB solution (Figure 2); and 3) Hyperosmolarity inhibits the glucagon release stimulated by both glucopenia and arginine (Figure 4-A).

Concerning the insulin and somatostatin release, stimulatory effects similar to the present results have been reported (Malaisse and Malaisse-Lagae, 1968; Gobena et al., 1974, Hermansen, 1982). The present study demonstrates that 3-OHB stimulates the insulin and somatostatin release even under the low glucose condition.

Concerning the glucagon release, Edwards and Taylor reported that 10 mM DL-3-OHB suppressed glucagon release from the isolated islets of guinea-pigs incubated in a medium containing 5.5 mM glucose (Edwards and Taylor, 1970), which is consistent with the present results. In the experiment to elicit the long term effects of ketoacidosis, the ketoacidotic perfusate inhibited glucagon release stimulated by glucopenia (2.8 mM glucose) and tended to inhibit it under the perfusate condition of 12.5 mM and 22 mM glucose, whereas it did not affect glucagon release under the perfusate condition of 22 mM glucose plus 10 mM arginine (Figure 4-A). The insulin release was not inhibited by this perfusion (Figure 4-B), suggesting that the inhibitory effect cannot be ascribed to its non-specific toxic effect on islet cells. On the other hand, it has been reported that the 3-OHB infusion in vivo does not have a clear effect on basal and hypoglycemia stimulated glucagon secretion in normal men and dogs (Sherwin et al., 1975; Müller et al., 1976; Quabbe et al., 1983). Namely, Quabbe et al. demonstrated that the infusion of 3-OHB alone (resultant plasma 3-OHB concentration was about 1 mM) caused a slight increase in basal plasma glucagon (10–20 pg/ml) and a delayed peak of plasma glucagon in response to insulin-hypoglycemia. These discrepancies might be ascribed to an offsetting action between the slight inhibitory effect of 3-OHB on glucagon secretion produced by glucopenia and its small stimulatory effect on glucagon secretion produced by arginine, in addition to the difference between in vitro and in vivo experiments and/or the difference in the 3-OHB concentration used.

The above results were obtained with
2.5–10 mM D-3-OHB which is sometimes observed in diabetic ketoacidosis (Müller et al., 1973) and prolonged starvation (Koeslag et al., 1982; Féry and Balasse, 1983). In prolonged starvation, the decrease in plasma glucose causes an increase in plasma glucagon. The present result suggests that increased 3-OHB during prolonged starvation and diabetic ketoacidosis might directly suppress glucagon release from pancreatic \( \alpha \)-cells and form a negative feedback system. However, in diabetic ketoacidosis hyperglucagonemia is brought about by more potent stimulants of glucagon release such as an absolute deficiency of insulin, stress and hypovolemia (Unger and Orci, 1976) although the present results suggest that ketoacidosis is not a factor in the vicious cycle of diabetic ketoacidosis.

Hyperosmolarity suppressed the glucagon release stimulated by both glucopenia (2.8 mM glucose) and arginine (Figure 4-A). On the other hand, insulin release measured in the same experiment was not influenced by the hyperosmolarity (Figure 4-B). Blackard et al. demonstrated a transient increase in insulin and glucagon release from perfused chopped rat pancreas following an abrupt reduction of medium osmolarity (Blackard et al., 1975). They also reported that an increase in medium osmolarity of 60 mOsm/kg with NaCl did not affect insulin release. However, there is no previous report concerning the effect of hyperosmolarity on glucagon secretion, and the present results are first demonstration of the suppressive effect of hyperosmolarity on glucagon secretion.

In diabetic ketoacidosis or non-ketotic hyperosmolar coma, the plasma glucagon concentration is extremely high in spite of concomitant hyperosmolar state. Therefore, the pathophysiological significance of this suppressive effect must be slight, although this effect might contribute somewhat to the cause of non-ketotic hyperosmolar coma in addition to hepatic unresponsiveness to glucagon, in terms of its ketone body production, due to aging (Okuda et al., 1987).

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**References**


