GLUCAGON AND INSULIN SECRETION DURING ACID-BASE ALTERATIONS

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Numerous studies have demonstrated that glucagon secretion is regulated by different metabolites, hormones and drugs.

Recently, in a previous report, we have demonstrated that modifications of the normal extracellular pH surrounding the islet cells from 7.4 to 7.8, result in increased arginine-induced glucagon secretion[14]. Conversely, this pH change impairs insulin secretion elicited by glucose, arginine or sulfonylureas[13,15,16].

The foregoing observations prompted us to explore in more detail A-cell behavior under extracellular alkalosis. The chosen experimental model was the perfusion of isolated rat pancreas.

The present report deals with the effect of sequential extracellular pH changes (7.4-7.8-7.4) on the response of A and B cells to hypoglycemia or an arginine stimulus. Furthermore, results of arginine-induced glucagon and insulin secretion under constant alkalosis are reported.

MATERIAL AND METHODS

Female Wistar rats weighing 180-220 g were anesthetized with sodium pentobarbital (48 mg/kg i.p.). After laparotomy, all the vessels connecting the pancreas with the spleen, stomach, duodenum, colon and small intestine were ligated together with both renal arteries. The whole organ was perfused through the celiac and superior mesenteric arteries via a cannula placed into the abdominal aorta. Exocrine secretion was drained through a small incision in the choledocus.

The perfusion system was similar to the one described by LECLERCQ-MEYER et al.[6] with minor modifications. To achieve quick substitutions of the pH 7.4

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buffer for the pH 7.8 ones or vice versa without stopping the experiments, two identical perfusion systems driven with a six channel peristaltic pump (Desaga) were connected by three-way stop-cock to a common cannula inserted in the aorta.

The perfusate consisted of Krebs-Ringer bicarbonate buffer containing 25 mEq/l CO_3H^-.

The pH 7.8 solution was obtained by partially replacing NaCl with $NaHCO_3$ to a final concentration of 60 mEq/l HCO_3^-. Thus Na^+ concentration and osmolarity were kept constant in both solutions.

The solutions were supplemented with 4% dextran (Sigma, average m.w. 79,000)-0.5% bovine serum albumin (Sigma) kept at 37 °C and equilibrated continuously with a gas mixture of 5% CO_2-95% O_2. Glucose concentration was 2.3 mM or 8.3 mM.

The pH values in the perfusion solutions were recorded continuously with a glass electrode immersed in the solutions equilibrated with the gaseous phase.

The flow rate was 1.90-2.00 ml/min. The perfusate, after a single passage through the pancreas, was collected every minute from a portal cannula into chilled tubes containing $EDTANa_2$ (1.3 mg/tube) and aprotinin (1,500 U/tube), immediately frozen and kept at −20 °C until glucagon and insulin assay. Pressure in the perfusion system averaged 25 ± 3 mmHg.

L (+)-arginine solution was infused at a rate of 0.09 ml/min through a side-arm pump-driven syringe into the perfusion solution to attain a final 5 mM concentration.

In a series of experiments the pancreases were initially exposed to 2.3 mM glucose in a pH 7.4 buffer during 30 min to establish basal glucagon and insulin secretion rates. Then the pH 7.4 medium was switched to another of pH 7.8, during 20 min and again to pH 7.4 for the final 20 min (fig. 1-A_1). In the corresponding control experiments the two perfusion systems, both pumping the same pH 7.4 buffer were connected to the pancreases alternatively at the same time intervals as before (fig. 1-A_2).

Two different experimental protocols were used in the experiments with arginine as a test substance. In both, glucose concentration throughout was 8.3 mM. In the first series, 5 mM arginine was added to the perfusate during 52 min, at pH 7.4, except between min 20 to 40 when the pH was 7.8 (fig. 1-B).

In the second series, 8.3 mM glucose was present throughout the experiments, the pH was also kept constant at a value of 7.4 (fig. 1-C_1) or pH 7.8 (fig. 1-C_2). The test substance, 5 mM arginine, was applied during a 20-min period starting at 0 min.

Glucagon and insulin were assayed with charcoal-dextran methods[2,18].

Glucagon antiserum 30 K together with purified ^{125}I-glucagon prepared according to JØRGENSEN and LARSEN[5] and a porcine standard (Lilly lot 258-VO16-36) were used for glucagon assay. Insulin antiserum was kindly provided by Dr. R. Gutman.

Total calcium concentration in the solutions throughout the experiments was 2.25 mM. A diminution in proton concentration has been reported to increase calcium binding to albumin[10] and bicarbonate[17]. Thus, to determine the effective ionized calcium concentration in the solutions employed it was measured anaerobically at 37 °C with an ionized calcium analyzer (Orion

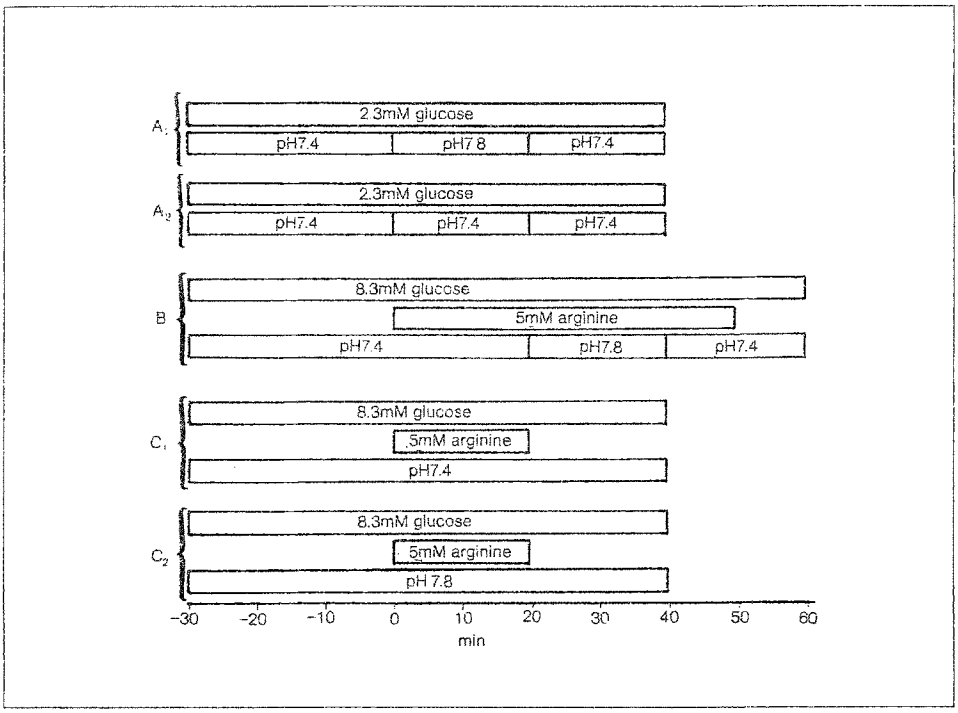

Fig. 1 - Schematic representation of the different experimental protocols used in rat pancreas perfusion. Details are described in 'Material and Methods'. Time of perfusion in minutes.

Space-State 20). The values of Ca^{2+} concentration (mM) in the buffer employed in the experiments were: 1.60 ± 0.05 (n = 4) for pH 7.40 ± 0.02 and 1.30 ± 0.05 (n = 4) for pH 7.80 ± 0.02; i.e., a 18.5% decrease in Ca^{2+} for a 0.4 pH unit increment.

When dextran is substituted by 4% bovine serum albumin, another solution commonly used in organ perfusion experiments, Ca^{2+} concentration (mM) averaged 0.75 ± 0.01 (n = 8) and 0.53 ± 0.01 (n = 5) at pH 7.4 and 7.8 respectively.

Statistical analysis of the data was performed by nonpaired *t*-test.

RESULTS

Effect of alkalosis on glucagon release in the presence of 2.3 mM glucose - Fig. 2-a shows the response of A-cells challenged with a constant perfusion of 2.3 mM glucose during three successive periods of pH 7.4, 7.8 and 7.4. The changes in extracellular pH in the preparations were achieved almost immediately due to the small dead space (0.5 ml) between the stop-cock connecting both buffer reservoirs and the aortic cannula.

During the first 30 min at pH 7.4, A-cells were stimulated and consequently mean glucagon output was 0.65 ± 0.14 ng/min.

Extracellular pH shift towards alkalosis produced an immediate and transient three-fold increase in glucagon output. This spike lasted 4 min

reaching its maximum within the first minute following the pH change. Thereafter, glucagon release did not show any special modification, not even when the pH returned to 7.4.

Change of the perfusate pH from 7.4 to 7.8 did not alter basal insulin secretion elicited by 2.3 mM glucose (fig. 2-b).

Control experiments with a constant 2.3 mM glucose concentration at pH 7.4 and the same maneuvers, alternatively connecting the two perfusion systems to the cannula placed into the rat aorta did not reveal either spikes of glucagon release (fig. 2-c), or modifications in insulin output (fig. 2-d).

Effect of alkalosis on arginine-induced glucagon and insulin release in the presence of 8.3 mM glucose - Fig. 3 shows the pancreatic release of glucagon and

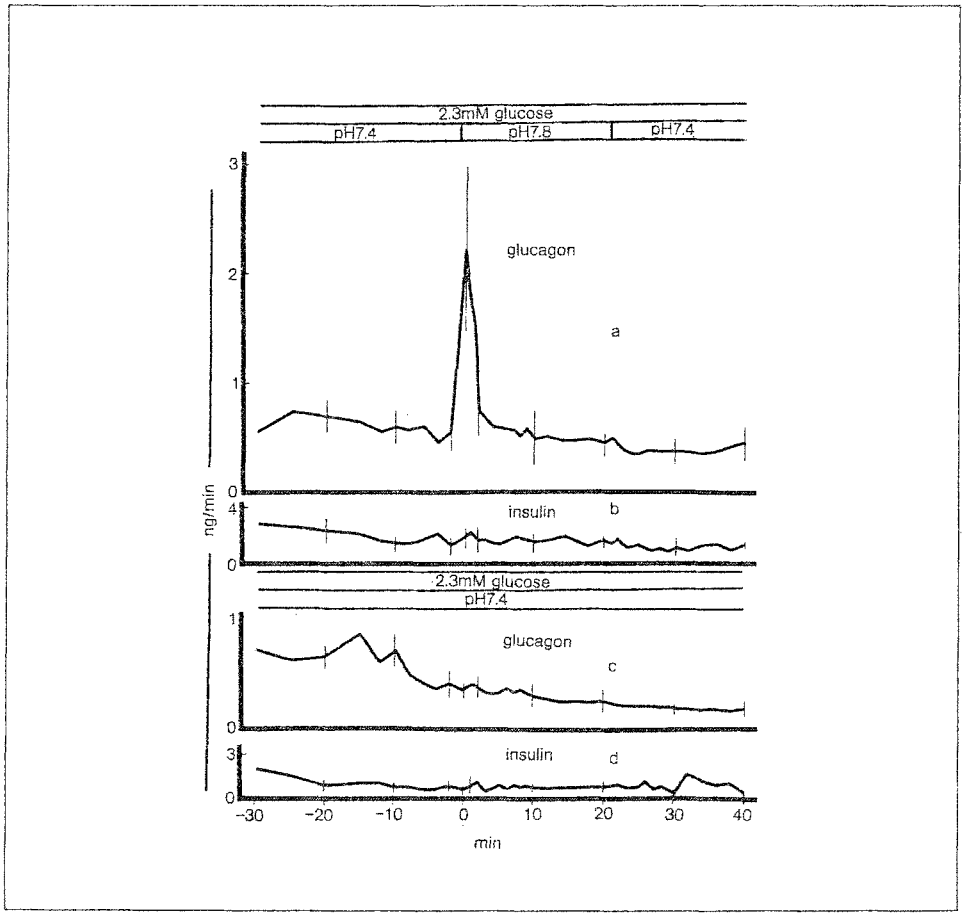

Fig. 2 - Influence of pH changes in the perfusion medium on glucagon (a) and insulin (b) secretion elicited by a constant 2.3 mM glucose concentration from the rat pancreas. After a 30 min period of pH 7.4, the medium was switched to pH 7.8 during 20 min, followed by a second 10-min period with pH 7.4 medium. In c and d, after the 30-min period of pH 7.4, the medium was switched to one of identical pH for a 20-min period. Thus the (c)- and (d)-perfusions served as switching controls. Secretory rates of glucagon and insulin ± SEM in ng/min of 7 perfusions for each panel are shown. Time of perfusion in minutes.

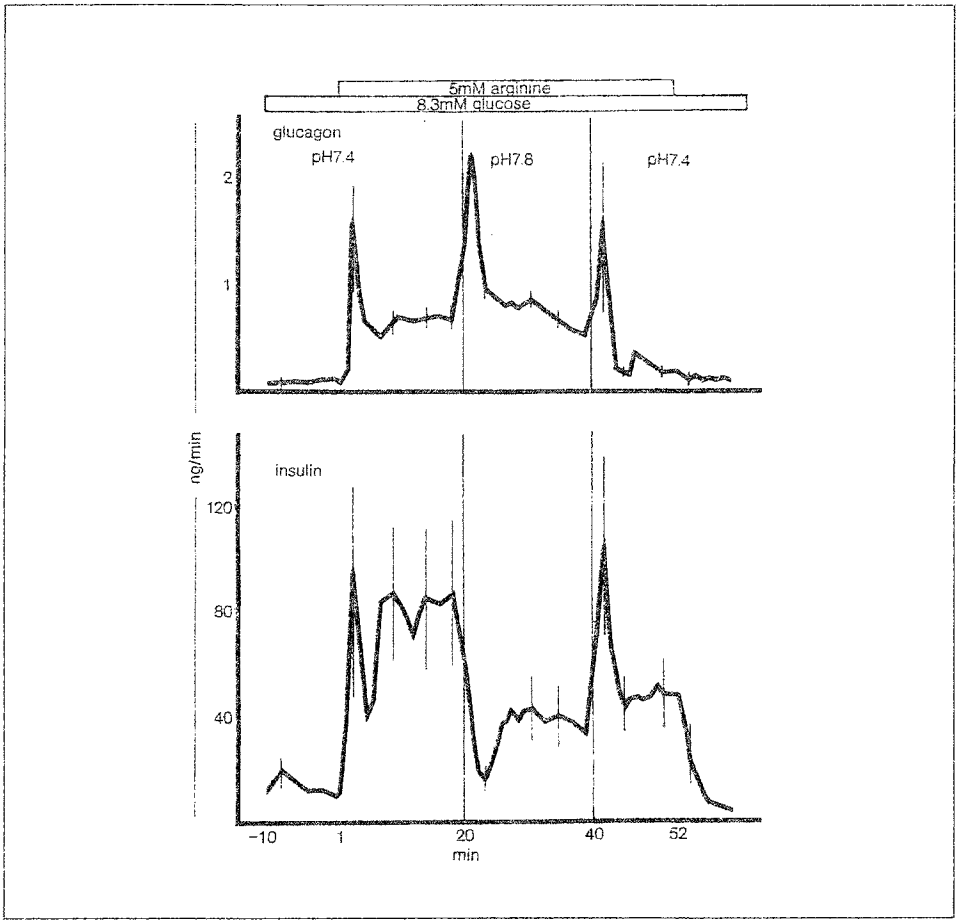

Fig. 3 - Influence of switching the pH in the perfusion medium from 7.4 to 7.8 during a 5 mM arginine stimulus, with constant 8.3 mM glucose concentration, on glucagon and insulin secretion from the perfused rat pancreas. Details of the experimental protocol appear in fig. 1. The values for glucagon (upper panel) and insulin (lower panel) release in ng/min are the means ± SEM of 9 experiments. Statistical analyses for differences in the hormone secretion rates appear in tab. 1.

insulin elicited by arginine during constant perfusion of glucose (8.3 mM) in three successive periods of pH 7.4,7.8 and 7.4.

For the first 30 min equilibration period, minute amounts of glucagon were found in the perfusates while insulin levels were easily detected as a consequence of the 8.3 mM glucose concentration in the medium (tab. 1).

During the first pH 7.4 cycle in response to the arginine stimulus, glucagon secretion showed the already known biphasic pattern.

When the pH of the perfusate was switched to pH 7.8, glucagon secretion showed an immediate and short burst with a fast return to previous values. Following the restoration of the pH to 7.4, a similar glucagon burst was observed but in this case with a return to significantly lower levels than after the response to pH 7.8.

time (min)	−30 to 1	2 to 18	20 to 38	40 to 52	54 to 68
pH	7.4	7.4	7.8	7.4	7.4
stimulus (mM)	Glu 8.3	Glu 8.3 Arg 5	Glu 8.3 Arg 5	Glu 8.3 Arg 5	Glu 8.3
glucagon (ng/min)	0.097 ± 0.017	0.692 ± 0.042*	0.948 ± 0.057**	0.418 ± 0.065$^{\Delta}$	0.082 ± 0.014
insulin (ng/min)	12.8 ± 1.2	72.6 ± 6.2$^{\nabla}$	35.7 ± 2.8^{O}	56.5 ± 5.3^{O}	7.0 ± 1.1

statistical analysis: * *vs* ** : $p<0.005$; ** *vs* $^{\Delta}$: $p<0.001$; * *vs* $^{\Delta}$: $p<0.005$; $^{\nabla}$ *vs* ^{C} : $p<0.001$; ^{C} *vs* ^{O} : $p<0.005$.

Tab. 1 - Mean secretion rates of glucagon and insulin in ng/min by perfused rat pancreas during the periods and conditions indicated above. Number of perfusions = 9.

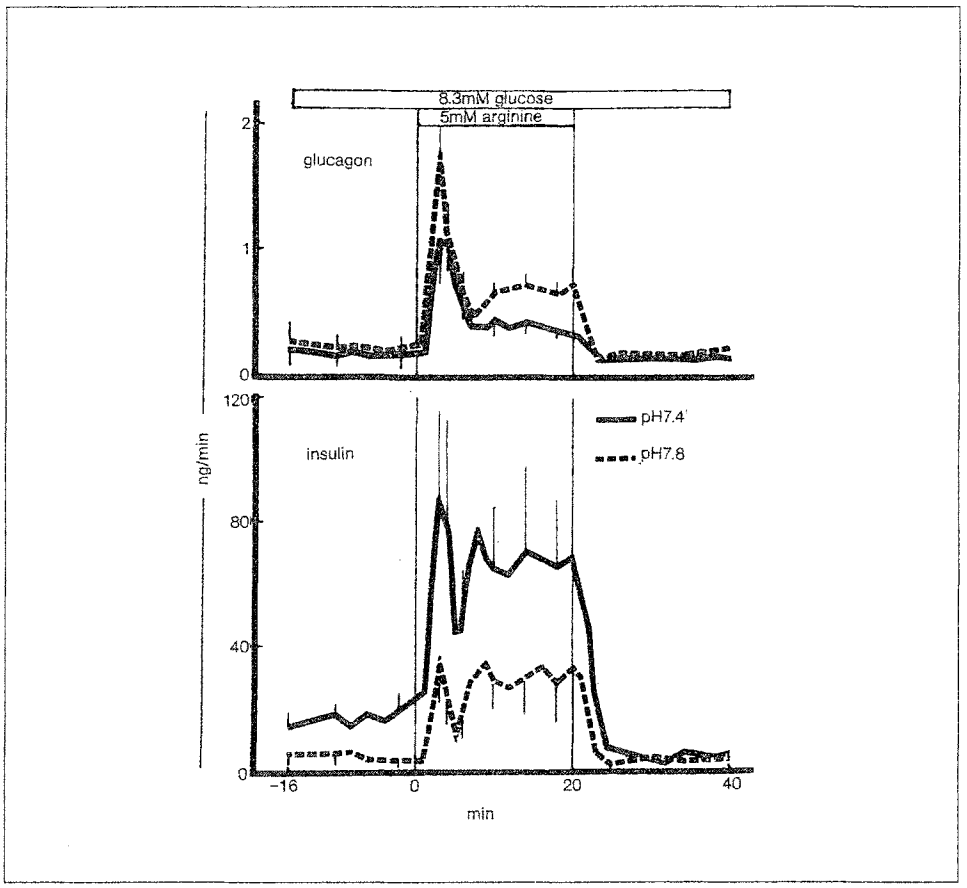

Fig. 4 - Influence of pH of perfusion medium on arginine-induced glucagon (upper panel) and insulin (lower panel) secretion. The pH of the perfusion media, 7.4 or 7.8, was kept constant throughout the experiments. Perfusions with pH 7.4 are represented by a solid line, and those with pH 7.8 by a dashed line. The arginine stimulus, present during 20 min, was started at time 0, 30 min after the onset of the experiments. Values for glucagon and insulin release are the means ± SEM in ng/min of 7 experiments for pH 7.4 and 6 for pH 7.8. Statistical analyses for differences in hormone secretion rates appear in 'Results'.

Thus, mean glucagon secretory rates obtained for each pH period exhibited the following relationship: pH 7.8 > 7.4 (first) > 7.4 (second) (tab. 1).

During the first period of pH 7.4, insulin, like glucagon, showed a biphasic secretory pattern. Conversely to what was observed for glucagon, the second phase of arginine-induced insulin secretion appeared significantly reduced when extracellular alkalosis was installed (tab. 1). This reduction was present throughout the 20-min period of alkalosis with a particularly marked diminution in insulin output during the first 5 min following the pH change. The inhibitory effect of alkalosis on insulin secretion was immediately and completely removed by switching the pH of the perfusate from 7.8 to 7.4. A biphasic insulin secretion pattern in response to arginine was again obtained; although the mean secretion rate compared to the initial pH 7.4 period was lower, it was not significantly different.

Figure 4 shows the effect of a 5 mM arginine stimulus present during 20 min on glucagon and insulin secretion at pH 7.4 and 7.8. In this case, the pH of the buffer and the glucose concentration (8.3 mM) were kept constant from the beginning of the experiments.

Under arginine stimulus at pH 7.8, the values for the first peak were larger than at pH 7.4 but only reached the level of significance during the second phase [mean values 0.629 ± 0.032 and 0.334 ± 0.022 ng/min, respectively ($p < 0,001$)]. In addition, if the total output is calculated as the areas under the curves during the total arginine period, glucagon output at pH 7.8 was significantly higher than at pH 7.4 [0.769 ± 0.059 ng/min and 0.520 ± 0.051 ng/min respectively ($p < 0.01$)].

The insulin release pattern at pH 7.4 and 7.8 already differed from the stabilization period (−30 to 0 min) (fig. 4). Indeed, the 8.3 mM glucose concentration employed exceeds the stimulatory threshold for the B-cells and consequently the inhibitory effect of extracellular alkalosis on the glucose-induced insulin secretion already described[16] becomes evident. This inhibition averaged 60% (6.2 ± 1.1 ng/min *vs* 17.2 ± 1.4 ng/min; $p < 0.001$).

Although changes in extracellular pH do not modify the biphasic pattern of arginine-induced insulin secretion, a 60% reduction in total hormone output was observed at pH 7.8. The mean secretory rates were 62.3 ± 5.3 ng/min at pH 7.4 and 24.8 ± 0.7 ng/min at pH 7.8 ($p < 0.001$).

DISCUSSION

The present results correlate with previous findings regarding the effect of changes in the pH of the extracellular fluid of islet cells on their secretory response elicited by several agents[13, 14, 15, 16].

Although the high pH was present from the onset of the experiment or following a perfusion cycle at pH 7.4, a 55% drop in the amount of insulin released in response to arginine was observed. As was previously shown with glucose-induced insulin secretion[12], this inhibition was immediately installed and removed according to the pH of the perfusate. Hence, the blocking effect of alkalosis is independent of the stimulatory agent employed.

Glucagon secretion in response to low glucose was increased when the extracellular pH shifted from normal to alkalosis. The recognition of the alkalotic state by A-cells was almost immediate but short-lasting. Moreover,

glucagon output remained unchanged when the reverse situation, the change from pH 7.8 to pH 7.4 occurred. This transient but significant alkalosis-induced glucagon output was detected using a high frequency sampling system. Hence, it might be possible that using another sampling method this phenomenon could easily be overlooked.

Arginine-induced glucagon secretion showed a strong dependence on extracellular pH. Contrary to the inhibitory effect of extracellular alkalosis on arginine-induced insulin secretion[15], glucagon output was significatively increased under this stimulus at pH 7.8 (figs 3 and 4, and tab. 1).

Moreover, in pancreas preparations previously equilibrated at pH 7.8, the introduction of the arginine stimulus produced a greater response than the one obtained at pH 7.4 (fig. 4). On the other hand, when the pancreas equilibrated at pH 7.4 was already under the effect of a stimulatory agent, either low glucose or arginine, a switch in pH to 7.8 produced a first and transient glucagon release.

However, A-cells were unable to maintain sustained secretion in response to arginine when the extracellular pH returned to 7.4 after a 20 min exposure to pH 7.8.

Different interpretations can be put forward to explain this behavior, e.g. the aging of the preparations after 70 min of perfusion. This is particularly true for A-cell secretion even under a potent hypoglycemic stimulus (see fig. 2-c). Another possibility is that as a result of exposure to pH 7.8 for a certain length of time, A-cells might become low responders to arginine at pH 7.4.

All these results demonstrate a completely opposite effect of extracellular alkalosis on insulin and glucagon secretion. Hence, this effect might represent a well-developed adaptive response of islet cells to the pH changes, rather than a deleterious effect of alkalosis on those cells.

Several hypotheses can be put forward to explain the results observed.

Calcium ion concentration is modified as a consequence of high pH[10, 17]. Alkalosis decreases ionic calcium activity in the perfusate due to the formation of non-ionized complexes of the cation with HCO_3^- and albumin, as has already been reported[13] and also observed in the present study. Nevertheless, it has been shown that reduced B-cell response to glucose is not overcome even if sufficient $CaCl_2$ excess is added to the pH 7.8 buffer to obtain a calcium ion concentration similar to that at pH 7.4[13].

In a similar way, it is not very plausible that the lower Ca^{2+} concentration during alkalosis may be responsible for the enhancement of glucagon response to glucose or arginine.

Modifications of glucagon secretion in the presence of high or low glucose concentrations have been described for normal and calcium-depleted media[1, 4, 7, 8, 20]. These extreme situations are different from the decrease observed in our conditions where Ca^{2+} diminished only by 18.5% from pH 7.4 to 7.8.

It has been reported by HUTTON et al.[3] that glucose- or α-ketoisocaproate (α-KIC)-induced insulin secretion in B-cells depends on the drop of intracellular pH caused by the secretagogues. According to their results, the optimum value for the secretion would be obtained with an intracellular decrease of 0.09 pH unit below the basal level. Consequently, the optimal extracellular pH for insulin secretion shifts to higher values when glucose or α-KIC concentration in the extracellular fluid are increased. Thus, in Hutton's experiments an

extracellular pH increase of 0.4 units beyond the apex, always resulted in lower insulin output, provided that for a 6.7 mM glucose concentration in the milieu the increase was considered from pH 7.0 to 7.4; for 11.1 mM glucose from 7.4 to 7.8 and for 27.8 mM glucose from 7.6 to 8.0.

In view of these observations, and with the evidence that metabolic fluxes regulate hormone release both in A- and B-cells[8,11] one could speculate that glucagon secretion might also be coupled to intracellular pH changes but in the opposite direction. Hence, the alkalotic state would be a more favorable situation for glucagon secretion than the normal pH of 7.4, with the glucose or arginine concentrations presently employed.

A possible paracrine interrelationship[19] modified by the pH shift from normal to alkalosis cannot be completely ruled out. In fact, glucagon output was increased with a concomitant fall in insulin secretion without evident modifications either in flow or in total pressure of the perfusion system.

In conclusion, these results suggest an important role for extracellular pH in the regulation of glucagon and insulin release. Furthermore, they provide new evidence for a reverse coupling of the secretory response of A-and B-cells to stimuli or experimental conditions. Although some possible mechanisms can be suggested to account for this kind of regulation, further study is necessary for a definite interpretation.

SUMMARY

Previously, we reported that change from the normal pH of 7.4 surrounding the islet cells to 7.8 results in a decreased B-cell response to 16.6 mM glucose, 10 mM arginine or 400 μg/ml tolbutamide. In the present report we studied the effect of modifications in the extracellular pH on glucose- and arginine-induced glucagon and insulin secretion by the perfused rat pancreas. It was found that at pH 7.8, arginine-induced glucagon secretion was significantly greater than at pH 7.4. On the other hand, the switch from pH 7.4 to 7.8 in a pancreas already stimulated by either low glucose or arginine, produced fast and transient glucagon release. Sequential extracellular pH changes from 7.4 to 7.8 and back to 7.4 in the presence of 8.3 mM glucose and a 5 mM arginine stimulus demonstrated that A- and B-cells rapidly modify their secretion in response to extracellular alkalosis in opposite directions. While glucagon output was enhanced (mean secretory rates at pH 7.4, 0.692 ± 0.042 ng/min and 0.948 ± 0.57 at pH 7.8), insulin secretion was clearly reduced (72.6 ± 6.2 ng/min and 35.7 ± 2.8 ng/min at pH 7.4 and 7.8, respectively). The above observations, together with our previously reported data, indicate that extracellular pH plays an important role in the regulation of glucagon and insulin release. Particularly, extracellular alkalosis enhances A-cell response to 2.3 mM glucose and 5 mM arginine while partially inhibiting B-cell secretion in the perfused rat pancreas.

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