Epinephrine Enhancement of Potassium-stimulated Immunoreactive Insulin Secretion

Role of Beta-adrenergic Receptors

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SUMMARY

Although epinephrine stimulates insulin release by activation of beta-adrenergic receptors, its dominant effect (mediated by stimulation of alpha-adrenergic receptors) is an inhibition of insulin secretion that is powerful enough to suppress the secretory activity of insulin's most potent stimulants. The insulin-secretory response to potassium chloride (KCl) infusion, however, is not suppressed; in fact, in ureter-ligated dogs simultaneously infused with 360 μg. epinephrine per hour and 2 mEq. KCl per kilogram per hour, insulin release is actually increased about threefold (over controls). Propranolol blockade of beta-adrenergic receptors essentially abolishes the insulin response to KCl infusion, with and without epinephrine. It is unlikely that KCl, like epinephrine, provokes insulin release by direct stimulation of the beta-adrenergic receptors of the beta cells of the pancreatic islets. However, potassium in some way enhances the beta adrenergic (secretory) activity of epinephrine and blunts its usually dominant alpha-adrenergic (inhibitory) effect. DIABETES 27:550-53, May, 1978.

Stimulation of immunoreactive insulin (IRI) secretion by exogenous glucose, glucagon, or tolbutamide administration is almost completely suppressed by simultaneous infusion of epinephrine.[1,2] This inhibition of insulin secretion by epinephrine has been extensively studied, in vivo and in vitro, with drugs that stimulate and block α- and β-adrenergic receptors. The studies confirm that epinephrine, by activating adrenergic receptors of the β cells of the pancreatic islets, has a dual effect on IRI secretion. Stimulation of β receptors provokes insulin release, while activation of α receptors inhibits it. In most previously reported situations, epinephrine exhibited a predominant α effect and blocked the action of substances that stimulate insulin secretion.[3]

The present set of experiments was originally designed to determine the influence of epinephrine on transmembrane potassium (K) transfer in ureter-ligated (UL) dogs made hyperkalemic by infusion of 2 mEq. KCl per kilogram per hour. Since KCl infusion stimulates IRI secretion,[4] such experiments afforded an opportunity to investigate the influence of epinephrine on K-stimulated IRI release. It was found that in UL dogs loaded with KCl, simultaneous infusion with epinephrine not only failed to inhibit insulin secretion but actually produced a striking enhancement of IRI release. Following propranolol blockage of β-adrenergic receptors, there was an almost complete inhibition of insulin secretion.

Infusion with 2 mEq. KCl per kilogram per hour results in a gradual increase of serum IRI concentration as serum K levels rise.[4,5] However, progressive hyperkalemia inevitably produces cardiotoxicity that terminates infusion experiments with ventricular fibrillation or asystole. In dogs that are UL about 20 minutes before infusion with 2 mEq. KCl per kilogram per hour, serum K rises quickly because of relatively poor transfer of infused K to intracellular fluid (ICF) and infusion must usually be discontinued in little more than an hour.[4] In UL dogs infused with KCl and epinephrine, however, improved transmembrane K transfer slows the rise of serum K and permits KCl infusion for from 2½ to 3¼ hours.

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METHODS

Data were compiled from 33 dogs of both sexes that weighed between 16.8 and 24.0 kg.; these were fasted for 18 hours before an experiment. All were anesthetized with sodium pentobarbital, 30 mg. per kilogram intravenously, and connected to a Harvard respirator. About 20 minutes before an experiment, a midline abdominal incision was made in 24 dogs and both ureters ligated. Depending on the experiment, one to three veins (the right jugular and the cephalic of each forepaw) were kept open by infusion of ~ 25 ml. per hour of 0.15 M NaCl. Four UL animals were infused for three hours with 360 μg. per hour of epinephrine (Adrenaline, Parke-Davis) to determine the validity and activity of this dose in ligated dogs. Twenty-nine intact and UL dogs were infused with various combinations of KCl, epinephrine, and propranolol through separate veins. Animals were loaded with K by discontinuing one of the forepaw NaCl infusions and replacing it with a Harvard peristaltic pump that delivered 30 ml. per hour of a KCl solution of such concentration that each dog received 2 mEq. per kilogram per hour. Similarly, epinephrine (360 μg. per hour) was delivered in 30 ml. of water. Beta-adrenergic receptors were blocked by administration (into the right jugular vein) of a priming dose of 5 mg. propranolol followed by an infusion of 5.0 mg. per hour in 30 ml. of 0.15 M NaCl; 15 minutes after administration of the priming dose, infusion of KCl (with or without epinephrine) was begun. During an experiment, dogs were connected with a Hewlett-Packard ECG machine and lead II was monitored at frequent intervals. KCl was administered until prelethal ECG changes appeared—ventricular bradycardia of < 30 beats per minute, ventricular flutter, or bizarre ventricular complexes (QRS).

The various protocols are detailed in table 1. In order to determine the influence of improved K transfer and of longer duration of infusion on the IRI response, the control animals for the epinephrine-treated ligated dogs included intact animals; in these, improved K transfer and urinary K loss retarded the rise of serum K and permitted the KCl infusion for about three hours before hyperkalemic cardiotoxicity halted the experiment,[5] approximately the duration of infusion in UL dogs treated with epinephrine.

Venous blood samples for the measurement of serum K and serum insulin were obtained from an exposed femoral vein immediately after anesthesia, before KCl infusion was begun, at appropriate intervals as it proceeded and when it was discontinued, i.e., when prelethal ECG changes appeared ("end point"). Serum K was determined with an Instrumentation Laboratory flame photometer with lithium as an internal standard, insulin by the method of Soeldner and Slone,[6] and blood glucose with glucose oxidase.[7]

RESULTS

The results in the four UL dogs infused with 360 μg. of epinephrine per hour for three hours indicate that this dose is as valid in UL dogs as it is in man.[1] Blood glucose levels increase almost threefold, with little or no changes in serum IRI concentration (figure 1). The IRI and K responses of the remaining 29 dogs are depicted in figures 2 and 3; included are only the values obtained when *all* of the dogs in a given group were alive and being infused. The ranges of the preinfusion and "end point" serum K values in groups A, B, and C were similar (4.1 to 4.6 and 9.4 to 10.9 mEq. per liter, respectively).

Figure 2 shows that the insulin response to KCl infusion alone in control UL and intact dogs (groups A_1 and A_2) results in an approximately fourfold rise of IRI, irrespective of the duration of the infusion (in UL animals, 60 to 86 minutes; in intact dogs, 172 to 200 minutes). Simultaneous epinephrine and KCl infusion in UL animals (B_1) raises IRI to a level approximately three times that of intact and UL controls, with increased IRI response at lower serum K levels. The longer duration of infusion (150 to 210 minutes) in B_1 (compared with the UL controls) stems from an increase of K transfer from extracellular fluid (ECF) to ICF. Both insulin and epinephrine are active in the increased K transfer (unpublished observation).

Figure 3 shows the insulin responses after propranolol blockade of β-adrenergic receptors. The

TABLE 1

Intact and UL dogs treated with various combinations of KCl, epinephrine, and propranolol

Group	n*	Surgery	Infusate KCl	Epinephrine	Propranolol
		Group A (control)			
A_1	5	UL	+	−	−
A_2	5	none (intact)	+	−	−
		Group B (experimental): epinephrine treatment			
B_1	7	UL	+	+	−
		Group C (experimental): propranolol treatment			
C_1	4	UL	+	+	+
C_2	4	UL	+	−	+
C_3	4	none (intact)	+	−	+

*Number of dogs

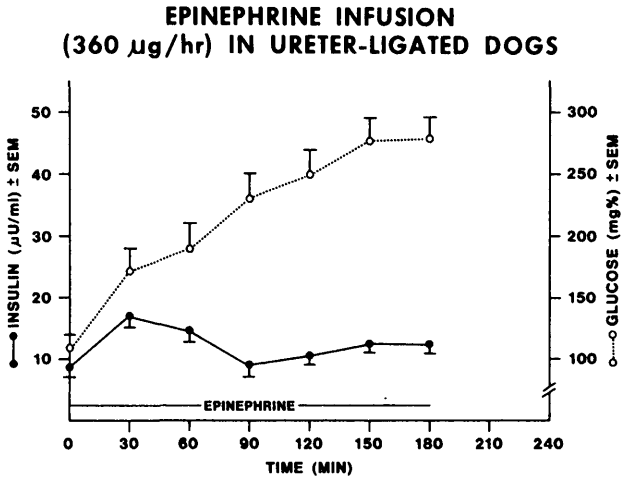

FIG. 1. Insulin and glucose responses in UL dogs treated with epinephrine. Vertical lines = S.E.M.

serum insulin curves for C_1 (UL, KCl, epinephrine) and C_2 (UL, KCl) are superimposable and are represented by a single graph. In group C, β blockade nearly obliterates the insulin response in each of the subgroups (C_1, C_2, C_3). (The graph of UL dogs treated with epinephrine and KCl and *no propranolol* (B_1) is included in the figure to emphasize the unequivocal inhibitory effect of β blockage.)

Usually the mean maximum change of blood glucose in each of the groups was near the end point, although this was less constant in groups C_2 and C_3. In group A the rise of serum IRI was consistently accompanied by a fall of blood glucose (mean of −20 mg./100 ml.). In B_1, in all animals, the striking rise of serum IRI was accompanied by a rise of blood glucose; mean glucose rose by 75 mg./100 ml. In propranolol-treated group C, serum IRI was low in all subgroups; mean blood glucose was relatively unchanged, except (as expected) in the animals treated with epinephrine (C_1), in which glucose rose by an average of 35 mg./100 ml.

DISCUSSION

The simultaneous infusion of epinephrine suppresses IRI release by almost all stimulants of insulin secretion.[3] However, the results of the present investigation indicate that hyperkalemia in UL dogs is nearly unique in that its secretory activity not only is not inhibited but is actually strikingly enhanced.

In control dogs (figure 2, groups A_1 and A_2), maximum serum IRI concentration is attained when serum K is close to the prelethal level (~ 9 mEq. per liter) and is either unchanged or somewhat diminished by further KCl infusion. On the other hand, in the experimental dogs of group B_1 (UL, KCl, and epinephrine), the concentration of serum IRI is similar to the maximum in controls, when serum K is only about 7 mEq. per liter; and IRI continues to rise, more abruptly than before, as KCl infusion continues to raise serum K to the prelethal "end point." At or near the "end point," the concentration of IRI in the serum is more than 10 times the preinfusion level and about three times the maximum response of controls in both those with brief infusions and those in which the duration of KCl infusion is about the same as that of experimental animals (figure 2). Also, ligation per se has no apparent effect on IRI response to KCl infusion—the response is similar in ligated and intact control dogs, although the duration of infusion differs considerably. It appears that the duration of infusion is not a factor in the different IRI responses of control and of the epinephrine-treated UL dogs (figure 2).

It is unlikely that hyperkalemia stimulates insulin secretion by direct activation of β receptors, although K-stimulated hormone release is almost completely suppressed by propranolol blockage (figure 3). Hyperkalemia could increase serum IRI by stimulating epinephrine secretion and obliterating its α effect by depolarization of the β cells of the islets; however, we have observed ample insulin secretion in adrenalectomized dogs infused with KCl (unpublished observation). On the other hand, KCl, like theophylline, may

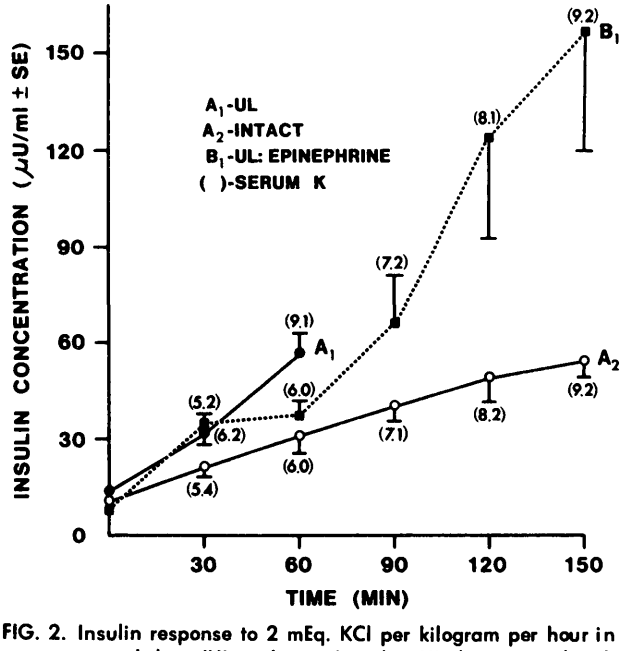

FIG. 2. Insulin response to 2 mEq. KCl per kilogram per hour in control dogs (UL and intact) and in UL dogs treated with epinephrine. Vertical lines = S.E.M.

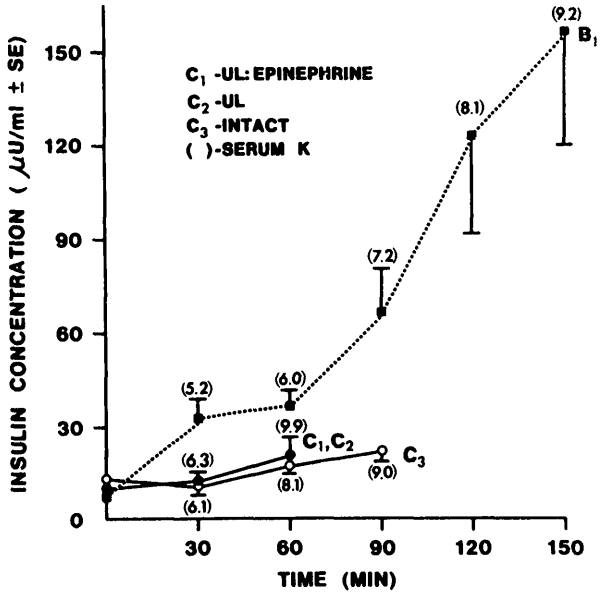

FIG. 3. Insulin response after propranolol blockade of β-adrenergic receptors in UL dogs treated with 2 mEq. KCl per kilogram per hour and epinephrine (C_1); also UL (C_2) and intact (C_3) dogs treated with KCl alone. Group B_1—UL, infused with KCl, epinephrine, and no propranolol—included for comparison. Vertical lines = S.E.M.

inhibit phosphodiesterase (enhance cyclic AMP activity) and thus increase the β adrenergic-receptor-stimulating effect of epinephrine in β cells of the islets. Theophylline-induced insulin release is also increased by epinephrine.[8]

It is, of course, possible that KCl infusion blocks α receptors and insulin secretion results from stimulation of β receptors by endogenous epinephrine, a response that would also be blocked with propranolol.[9] The copious IRI response to KCl infusion (figure 2) makes this an unlikely possibility, since the IRI response after phentolamine infusion in dogs is much shorter.[10]

The observations made in the course of this investigation suggest that K-epinephrine synergism may be responsible for the markedly enhanced IRI response in K-loaded UL dogs, a response that is considerably greater than the sum of the responses to each of the stimulants acting alone (figures 1 and 2).

The results presented are not consistent with the demonstration by Gomez and Curry that isoproterenol (an activator of β receptors) *blocks* K-stimulated insulin release in the isolated perfused rat pancreas.[11] Their study, however, entails a short burst of IRI that peaks in two minutes and returns to the basal level by 30 minutes—IRI that seems to be preformed hormone quickly released from β cells depolarized by K. In the present investigation, secretion of IRI is prolonged, apparently continuously synthesized by β cells. The different sources of the IRI or the species differences may account for the different findings.

Investigations of the insulin response to KCl and epinephrine were also carried out in intact animals. The insulin response, while not inhibited by epinephrine (it is similar to that of controls), is much less than in UL dogs. We have been able to demonstrate goodly amounts of urinary insulin, but a quantitative estimate of urinary IRI loss may not be accurate since radioimmune assay of urinary polypeptides is variably influenced by other constituents of the urine (personal communication).

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