Comparison of the Inhibitory Effects of Diphenylhydantoin and Diazoxide Upon Insulin Secretion from the Isolated Perfused Pancreas

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SUMMARY

Both diazoxide and diphenylhydantoin have been shown to cause hyperglycemia in man and to inhibit insulin secretion in vitro. The effects of these two drugs upon the response to 300 mg./100 ml. glucose were contrasted in the isolated, perfused rat pancreas. *Similarities*: Both drugs inhibited within seconds. At high concentrations (75 μg./ml.) of either drug, 95 to 100 per cent inhibition occurred. Upon withdrawal, return of secretion was rapid. *Differences*: During constant (five- and twenty-minute) infusions of diazoxide (10 to 75 μg./ml.), there was an initial fall in secretion and then an "escape" toward pre-inhibition levels; after diazoxide there was a postinhibitory overshoot. In contrast, five-minute infusions of diphenylhydantoin (5 to 75 μg./ml.), and twenty-minute infusions of 25 to 75 μg./ml., though causing comparable levels of initial inhibition, did not cause escape or postinhibitory overshoot. However, an incompletely inhibitory concentration of diphenylhydantoin (10 μg./ml.), given for twenty minutes, was followed by this overshoot.

Computer simulation, based on the compartmental-quantal model, suggested that diphenylhydantoin inhibits *both* secretion from a labile insulin compartment *and* provision of insulin and/or precursor to this compartment. Lower, partially inhibitory concentrations (i.e. 10 μg./ml.) of diphenylhydantoin might allow for some provision to proceed, and thus a postinhibitory overshoot would occur. The escape and overshoot noted with all inhibitory levels of diazoxide, when compared with the simulations, suggested that this drug may act primarily to inhibit a late step in the provisionary phase. DIABETES *21*:856-62, August, 1972.

For the past decade, both diazoxide and diphenylhydantoin (DPH) have been known to produce hyperglycemia in man.[1-11] In vitro studies have shown that diazoxide can inhibit insulin secretion,[12-16] and that this inhibition accounts, in part, for its hyperglycemic actions. We have described the inhibition of insulin secretion by DPH in the in vitro, perfused rat pancreas.[17] In this same system, Grodsky et al.[18-20] have noted that glucose infusion produces a diphasic release of insulin; a rapid early discharge, followed by a later phase, characterized by gradually increasing secretion.

In the current study we have compared DPH and diazoxide with respect to their effects upon the late phase of insulin secretion. Differences in the type of inhibition by the two agents were kinetically evaluated using a computer, by comparison with theoretical responses derived from a compartmental-quantal model.[21]

MATERIALS AND METHODS

The technic for perfusion and isolation of the rat pancreas has been previously described.[22] Male, Long-Evans rats, weighing 300 to 350 gm., were fasted overnight. The pancreas, upper duodenum, stomach and spleen were dissected from the anesthetized animals. This preparation was perfused with Krebs-Ringer buffer and 4 per cent human albumin,* utilizing a constant temperature control and oxygenation system. Glucose

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*Generously provided by Drs. M. Mozen and A. R. Pappenhagen of the Cutter Laboratories, Berkeley, California.

and/or inhibitors were added via a sidearm syringe. Glucose and drug levels were controlled by mixing weighed amounts of these substances into the sidearm syringes and infusing them at rates calculated to give the desired dose. Insulin assay was performed by the method of Grodsky and Forsham.[23]

Experimental design

1. *Twenty-minute mid-hour infusions.* In this type of experiment, the pancreas was perfused with glucose, 300 mg./100 ml. for sixty minutes. During the second twenty-minute period (twenty to forty minutes), diazoxide* or diphenylhydantoin (DPH) was infused at a constant concentration. The concentrations of DPH used were 10, 25, and 75 μg./ml. The concentrations of diazoxide used were 25 and 75 μg./ml.

2. *Sequential, five-minute infusions.* Glucose, 300 mg./100 ml., was continuously infused for sixty minutes. In separate experiments, the inhibitors were given in levels of 5, 10, and 15 μg./ml. at 20 to 25, 35 to 40, and 50 to 55 minutes, respectively. In other experiments, higher concentrations of the inhibitors (25, 50, and 75 μg./ml.) were added at similar time periods.

*Generously provided by Drs. I. I. A. Tabachnick and A. Gulbenkian of the Schering Corp., Bloomfield, N.J.

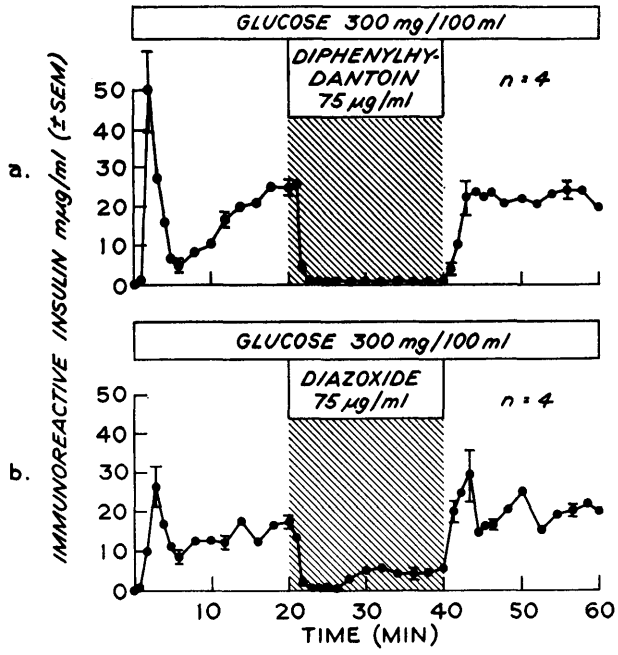

FIG. 1. Responses to diphenylhydantoin (a) and diazoxide (b), 75 μg./ml. of each drug, in the presence of glucose 300 mg./100 ml.

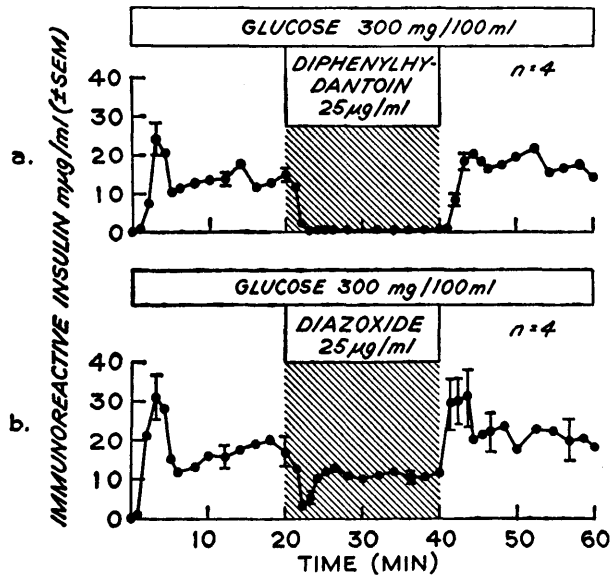

FIG. 2. Responses to diphenylhydantoin (a) and diazoxide (b) 25 μg./ml. of each drug, in the presence of glucose 300 mg./100 ml.

RESULTS

Immunoassay for insulin. Neither drug, at concentration of 75 μg./ml., influenced the standard curve for immunoassay of insulin.

Response to glucose, 300 mg./100 ml. In all experiments, a typical early discharge was followed by a fall and then a late rise in insulin secretion. This standard response[18-20] to glucose was modified by the inhibitors as noted.

1. *Twenty-minute mid-hour infusions (figures 1 through 3).* At a concentration of 75 μg./ml. (figure 1a), DPH inhibited insulin to undetectable levels. This inhibition began after one minute and remained con-

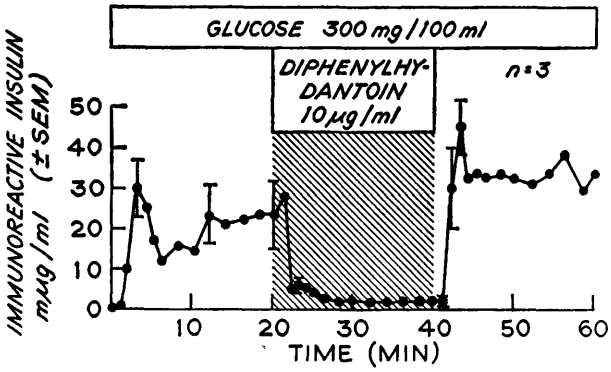

FIG. 3. Response to 10 μg./ml. of diphenylhydantoin in the presence of glucose, 300 mg./100 ml.

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stant throughout the drug infusion. Upon discontinuation of DPH, insulin secretion promptly returned to levels similar to those seen immediately prior to the infusion of this inhibitor. When diazoxide, 75 μg./ml., was infused (figure 1b), inhibition was again immediate and complete. However, after seven minutes an "escape" was suggested, during which low levels (3 to 6 mμg./ml.) of insulin secretion were detectable. Thus, the degree of inhibition by diazoxide changed with time, although diazoxide concentration was constant. After discontinuing diazoxide, return of insulin secretion was more rapid than with DPH (p-value for forty-one minutes, DPH vs. diazoxide $= < .01$); there was an "overshoot", followed by a fall, and then a gradual increase in secretion rates comparable to that normally seen with this concentration of glucose.[11]

At 25 μg./ml. DPH (figure 2a), the response was similar to that seen with 75 μg./ml., i.e. complete inhibition, then return of detectable secretion only after withdrawal. With diazoxide, 25 μg./ml., (figure 2b) strong inhibition was only transient, with "escape" occurring within four minutes after introduction of this drug. Insulin secretion stabilized at 10 to 13 mμg./ml. After discontinuing the drug there was again an overshoot, a fall, and a return of insulin secretion to pre-inhibitory levels.

When DPH, 10 μg./ml., was used (figure 3) inhibition of insulin secretion was again rapid. However, inhibition was not complete, with a fall to 6 mμg./ml. There was no "escape" pattern noted. After discontinuing this concentration of DPH there was a suggestion of an overshoot at forty-three minutes.

2. *Sequential, five-minute inhibitory infusions (figures 4 and 5).* Short infusions of DPH (5, 10, 15, or 25, 50, 75 μg./ml.) (figures 4a and 5a) produced early inhibition and constant, low levels of insulin secretion. This inhibition was partial, but definite, at 5, 10, and 15 μg./ml. and complete at 25, 50, and 75 μg./ml.

On the other hand, inhibition was not evident with diazoxide, 5 μg./ml. (figure 4b). However, higher concentrations [10, 15 (figure 4b), and 25, 50, and 75 μg./ml. (figure 5b)], produced prominent early falls in insulin secretion with escapes during infusion of the inhibitor, and postinhibitory "overshoot."

Table 1 statistically summarizes the phenomena of "inhibition", "escape" and "overshoot" observed with five-minute infusions of diazoxide in figures 4b and 5b, at drug concentrations ranging over 10 to 75 μg./ml. The nadir in insulin secretion (initial inhibition), seen two minutes after starting the diazoxide, was significantly less than it was prior to infusion of this drug. At 10, 15, and 25 μg./ml., insulin secretion rose from ini-

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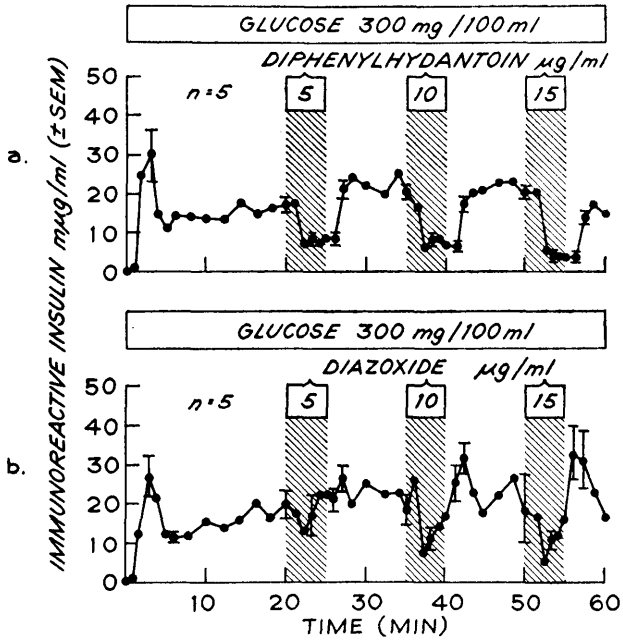

FIG. 4. Responses to short, sequential infusions of low concentrations of diphenylhydantoin (a) or diazoxide (b) in the presence of glucose, 300 mg./100 ml.

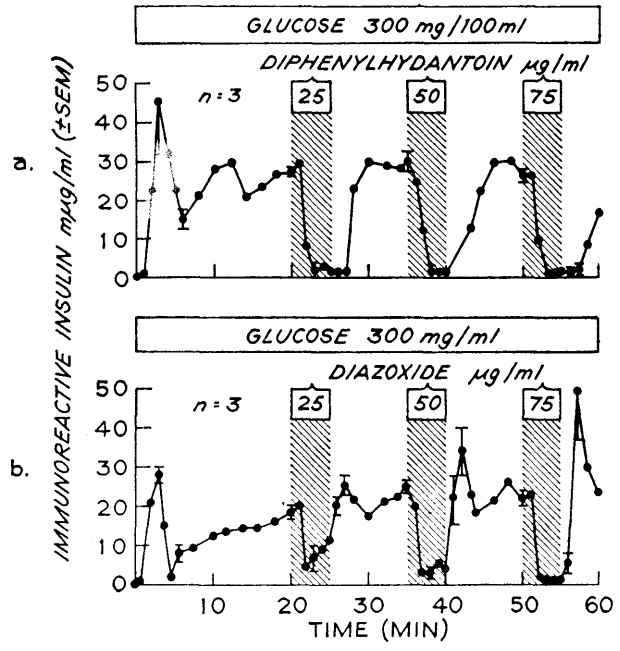

FIG. 5. Responses to short, sequential infusions of moderate concentrations of diphenylhydantoin (a) or diazoxide (b) in the presence of glucose, 300 mg./100 ml.

TABLE 1

Statistical assessment of observed responses to five minute diazoxide infusions, using 300 mg./100 ml. constant glucose stimulus

Drug concentration (μg./ml.)	No. of experiments*	Mean immunoreactive insulin concentration (mμg./ml.) a. Pre-inhibition level (1 min. prior to diazoxide infusion)	b. Initial inhibition (After 2 min. of diazoxide infusion)	c. Escape (Last min. of diazoxide infusion)	d. Overshoot (2 min. after discontinuing diazoxide)	e. Postovershoot (4 min. after discontinuing diazoxide)
10	5	18	8	17	33	12
15	5	18	5.8	16	31	16
25	3	18	5.2	12	25	17
50	3	25	4.6	5.3†	37	18
75	3	22	2.4	0.5†	48	20
	Mean	20.2	5.2	15.0	34.8	16.5
	S.E.	± 1.43	± .91	± 1.53	± 3.8	± 1.3
	p value	<.001	<.001		<.01	
		<.01				
		N.S.				
	Per cent of pre-inhibition level	100%	25%	74%	170%	81%

* See figures 4b and 5b.

† Escape from inhibition was not noted after five minute infusions of higher concentrations (50 and 75 μg./ml.) of diazoxide. Thus, insulin levels at these concentrations were not used for statistical evaluation of escape.

tial inhibitory levels of 25 per cent to 74 per cent of pre-inhibitory values (escape). Two minutes after discontinuing diazoxide, a secretion peak (overshoot) was seen which was significantly increased (170 per cent) over pre-inhibitory values. After the postinhibitory overshoot, insulin concentration returned to levels not significantly different from those seen in the pre-inhibitory period.

DISCUSSION

DPH inhibits insulin secretion in vitro.[17,24] In the present experiments we have contrasted this inhibitory effect with a well known inhibitor of insulin secretion, diazoxide. The structure of these two substances is shown in figure 6. There are no similarities in structure.

Similarities in inhibitory action of the two agents were that, at high concentrations (75 μg./ml.), they both caused 95 to 100 per cent inhibition of insulin secretion. Thus, when compared to diazoxide, on a weight and/or a molar basis, DPH is a potent inhibitor of insulin secretion. *Differences* noted were that diazoxide, at all inhibitory concentrations, effected an initial fall in secretion and then "escape" toward pre-inhibition levels, with a postinhibitory "overshoot," i.e. the rapid attainment of a transient, high secretion rate which, for a minute or two, exceeded pre-inhibitory levels. In contrast, DPH produced constant levels of inhibition during infusion, without escape and, except at partially inhibitory levels (10 μg./ml.), without significant postinhibitory overshoot.

From earlier experimental observations in the perfused rat pancreas,[19] insulin has been hypothesized to be stored in a labile compartment, responsible for the early peak of insulin release which is concentration-related to a larger, more stable storage compartment (figure 7). This latter compartment contains 98 per cent of total beta cell insulin. A provisionary effect (P) modifies the input of precursors, substrates, energy, and/or preformed insulin, resulting in more insulin being made available for secretion through the labile com-

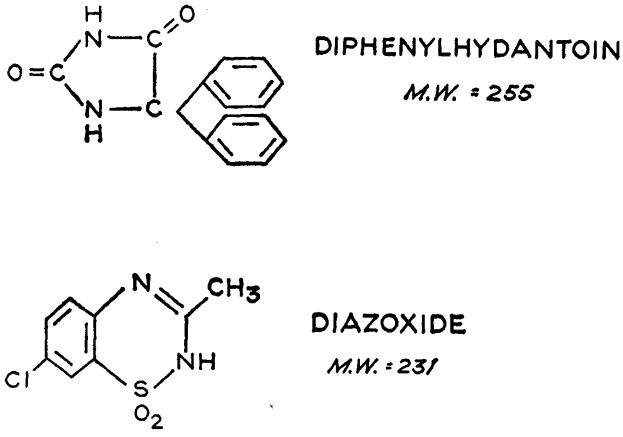

FIG. 6. Structures of diphenylhydantoin and diazoxide.

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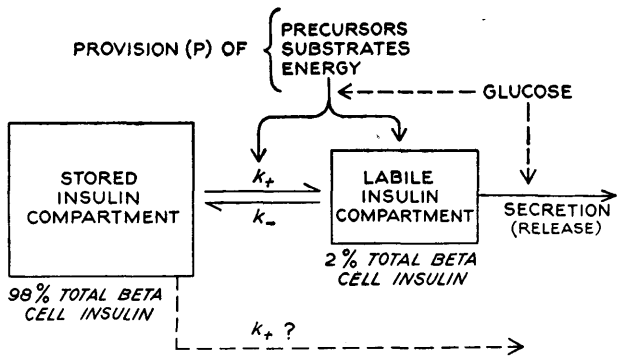

FIG. 7. Two-compartmental model for insulin secretion.

partment, with a resultant late phase of insulin secretion. Exchange coefficients (k_+ and k_-) characterize the rate of movement of insulin between the storage and labile compartments.

Theoretically, inhibitors acting at different sites in this system produce different secretion patterns. Thus it is possible to evaluate the mechanism of an inhibitor by comparing experimental patterns with theoretical curves.

Computer simulation,* using a compartmental-packet model[19,34] indicates that, in the presence of glucose, 300 mg./100 ml., inhibition of both release from and provision to the small compartment (figure 8a) would be characterized by (1) a rapid initial inhibition, (2) constant inhibitory rate maintained throughout infusion of inhibitor, without "escape", and (3) a return to pre-inhibitory rates of secretion, without "overshoot", after discontinuing the inhibitor. This response was seen with DPH in moderate to high concentrations. At very low DPH levels (10 μg./ml.), where inhibition of secretion was incomplete, some overshoot occurred. This suggests that at these drug levels, inhibition of the provisionary phase was less than at higher levels.

Similar computer simulation indicates that, with a 300 mg./100 ml. glucose stimulus, partial inhibition of release from the small compartment, but little or no inhibition of provision would theoretically (figure 8b) be characterized by (1) rapid initial inhibition, (2) an "escape" as increasing amounts of insulin enter the small compartment, providing additional insulin to the release system, and (3) a postinhibitory overshoot as that insulin, accumulated in the small compartment, is suddenly free to be released. This pattern is quite similar to that seen with diazoxide. However, the overshoot in our experiments was far less than depicted by the model. This suggests the alternate possibility that

*PDP-12 Digital Computer using FOCAL-1969 language.

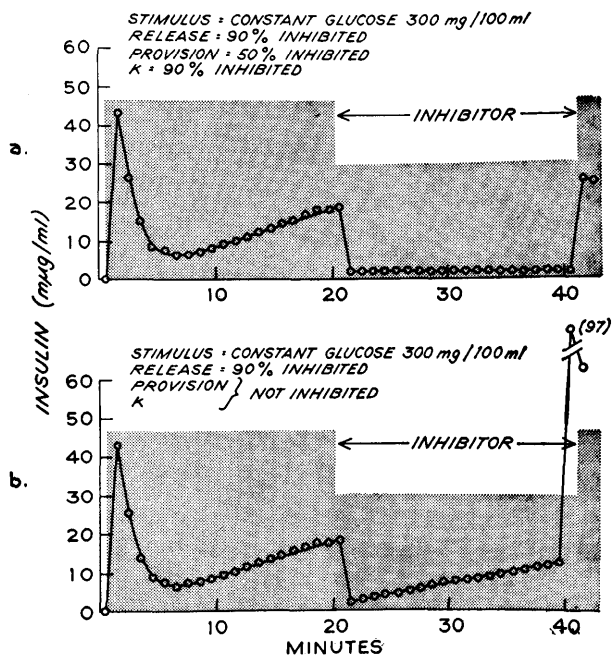

FIG. 8. Computerized simulations postulating inhibition of secretion (release) of insulin and other intracellular processes (a), and inhibition of secretion (release) only (b).

diazoxide inhibits a late step in provision. This would result in initial inhibition of provision-dependent release, then escape as accumulation occurs behind the blockade. Drug withdrawal would result in release of accumulated provisionary material and a modest, but definite, overshoot would occur.

Basabe et al.[16] described increased late phase secretion from pancreases of rats pretreated with diazoxide or its analog AO25 and concluded that these drugs inhibit insulin release but have no effects on insulinogenesis. These conclusions are consistent with our findings of escape and postinhibitory overshoot, which we suggest are due to continued provision but with an inhibition at a late step in the provisionary phase. When studying the inhibition of the response to glucose, Basabe et al. used very high diazoxide concentrations (150 to 300 μg./ml.). They failed to note the "escape" of insulin secretion observed by us with lower diazoxide levels, approaching those seen in patients taking the drug.[25]

Loubatieres et al.,[15] using 100 μg./ml. of diazoxide, demonstrated inhibition of the insulin secretory response to 150 mg./100 ml. glucose in the isolated perfused pancreas. Examination of their graphs reveals a suggestion of rapid escape from inhibition.

The escape and overshoot seen in our preparation is reminiscent of the secretory patterns seen with epine-

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phrine by Seltzer and Crout[25] in the intact dog. Interestingly, Burr et al.[26] recently have performed experiments in the perifused rat pancreas which demonstrated other similarities between diazoxide and epinephrine. Infusion of either substance prior to glucose enhanced the subsequent response to the carbohydrate stimulus. This enhancement is consistent with our "overshoot" phenomenon. These authors suggested that some of the effect of diazoxide may be mediated through the alpha and beta adrenergic systems. A possibly related observation was made recently by Hellman,[27] who noted that inhibition of insulin release by epinephrine or diazoxide was associated with a block between fructose 1, 6-diphosphate and 3-phosphoglycerate. There was accumulation of fructose 1, 6-diphosphate as well as other intermediates of glucose above this metabolic stage. Release of this substrate accumulation by sudden removal of diazoxide could result in an "overshoot" of insulin secretion, as we have observed, when these intermediates are rapidly metabolized.

Although the foregoing experiments and earlier studies[12-14,29] have demonstrated inhibition of insulin secretion by diazoxide, it has only recently been shown that diphenylhydantoin (DPH) inhibits the secretion of this hormone.[17,24] Kizer et al.[24] have shown that inhibition of insulin secretion by DPH cannot be overcome by tolbutamide. However, diazoxide inhibition can be reversed by tolbutamide.[14,25,28] This, in addition to our demonstration of basic differences in inhibitory patterns with these drugs, indicates that they act on different sites as well as mechanisms involved in eventual release.

DPH has been shown to reduce the uptake of sodium by isolated pancreatic islets.[24] Optimal sodium concentration is necessary for insulin secretion.[33] Since DPH can stimulate the sodium-potassium-magnesium ATPase-related "pump",[31] it seems possible that effects on cellular sodium are, in part, involved in the inhibitory mechanism of DPH. This "pump" system, in turn, may influence calcium uptake, an initial requirement for insulin secretion.[35,36]

Since alpha and beta adrenergic stimuli may be related to diazoxide's action,[26] and effects upon ATPase may cause some of the actions of DPH,[24,31] a relationship between the adrenergic and the ATPase systems is suggested. However, the nature of such a relationship has not been defined.

Finally, in contrasting DPH and diazoxide the following points are to be considered: (1) The qualitative insulin inhibitory patterns are sensitive to the concentration of these agents. (2) Inhibition by these agents may not be simply equated on a dose basis since they appear to act on different phenomena affecting release. Therefore, they produce divergent insulin inhibitory patterns with time. (3) Of the two, DPH has broader action, affecting both provision and release. This has been applied recently in studies of insulin secretion in two patients with insulinoma.[30]

ACKNOWLEDGMENT

The authors wish to thank Dr. Peter Forsham, for his helpful advice, Dr. Vochtek Licko, for cooperation and help with computer data, Mrs. Mary Ann Jones, Miss Florence Schmid, Miss Elizabeth Lewis, for excellent technical assistance, and to Mrs. Dorothea Faber for careful editorial assistance. This study was supported, in part by the Levi J. and Mary Skaggs Foundation and grants AM01410 and AM12763 from the U.S. Public Health Service.

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