The In Vitro Inhibition of Insulin Secretion by Diphenylhydantoin

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ABSTRACT Glucose intolerance has been observed following diphenylhydantoin (DPH) intoxication. Because of this association between DPH and hyperglycemia, the effect of DPH on insulin release in vitro using preparations of isolated islets of Langerhans and pancreatic pieces was examined. In concentrations identical with those considered necessary for adequate anticonvulsant therapy in man, DPH markedly decreases the insulin secretory response of pancreatic pieces to methacholine, 1 μ g/ml, tolbutamide, 250 μ g/ml, and glucose, 200 mg/100 ml, without any demonstrable alteration in the oxidative conversion of glucose-1-¹⁴C or glucose-6-¹⁴C to ¹⁴CO₂ by isolated islets. This DPH-induced inhibition of insulin secretion is not reversed by higher concentrations of glucose (600 mg/100 ml) or by increasing concentrations of extracellular calcium ion (4-6 mmoles/ liter). 0.1 mm potassium and 10⁻⁴ m ouabain, however, effectively restore the DPH-induced block of insulin secretion in pancreatic pieces. 60 mm potassium ion, on the other hand, not only restores the insulin secretory response to glucose (200 mg/100 ml) but results in an added stimulation of insulin secretion in the presence of DPH. In the presence of DPH, ²²Na accumulation by isolated islets is decreased by 26-40% as compared with controls. Such evidence is considered to indirectly support the postulate that the electrophysiological properties of DPH on the pancreas are due to a stimulation of the membrane sodium-potassium-magnesium ATPase.

INTRODUCTION

In 1965 an association between hyperglycemia and diphenylhydantoin (DPH) was described in rabbits (1). This observation was soon followed by the clinical recognition of hyperglycemia and nonketotic hyperosmolar coma resulting from DPH intoxication (2, 3), particularly in the critically ill or azotemic patient (3). The etiology of the DPH-induced hyperglycemia has been clarified somewhat by the demonstration of a transiently abnormal glucose tolerance test and low insulinogenic index in a patient with DPH intoxication which fully resolved after cessation of anticonvulsant therapy with DPH and reinstitution of treatment with phenobarbital (4). Millichap has studied the hyperglycemic effects of DPH and phenobarbital in rabbits and has found significant elevations of blood glucose within 1-2 hr after intraperitoneal administration of these agents (5), DPH being the more potent of the two drugs (5). From these data, Millichap has postulated that anticonvulsant therapy may stimulate the hypothalamus, resulting in hyperactivity of the sympathoadrenal system and subsequent hypoinsulinemia (5).

In this paper, studies are presented which further elucidate the mechanism of DPH-induced hyperglycemia and hypoinsulinemia. Studies were carried out on the effects of DPH on insulin secretion in preparations of isolated islets of Langerhans and pancreatic pieces. We have found that DPH and mesantoin, another hydantoin anticonvulsant, are potent inhibitors of pancreatic insulin release, whereas phenobarbital and hydroxydiphenylhydantoin, a metabolite of DPH lacking anticonvulsant properties, have no insulin inhibitory effects.

METHODS

Animals. Adult male Sprague-Dawley rats were obtained from Camm Research, Norfolk, Va. Animals were fed ad libitum a diet consisting of water and Purina Lab Chow up to the time of their sacrifice. For the isolation of islets, rats weighing more than 450 g were used, whereas animals weighing approximately 300 g were used for experiments involving whole pancreatic pieces.

Buffer. Fresh buffers were made daily and contained the following, unless otherwise specified: 100 mM sodium chloride, 5 mM potassium chloride, 2 mM calcium chloride, 1.25 mM KH₂PO₄, 1.25 mM magnesium sulfate, 26.2 sodium bicarbonate, 5 mM sodium pyruvate, 5.5 mM sodium fuma-

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rate, 5 mM sodium *l*-glutamate, and dextrose, 200 mg/100 ml. After preparation buffers were gassed 10 min with a 95:5 mxture of oxygen and carbon dioxide. In those experiments requiring the measurement of insulin secretion, bovine serum albumin was added to a final concentration of 0.2%. In those experiments requiring potassium in altered concentrations, NaCl and NaH₂PO₄ were either substituted for or replaced by KCl and KH₂PO₄ as necessary. All solutions were kept stoppered and on ice prior to use.

Piece experiments. For all experiments with pancreatic pieces except experiment set II, three to four rats were stunned by a blow on the head followed by cervical dislocation. The splenic portions of the pancreases were immediately removed and transferred to a glass Petri dish of buffer over ice. All visible fat and lymph nodes were removed, and the pancreas was cut into approximately 100 pices, each weighing about 5 mg. We have found that the use of smaller pieces markedly enhances the insulin output per milligram of tissue. The pancreas pieces were randomized, and approximately six to seven pieces were transferred to 10-cc Erlenmeyer flasks containing 2 ml of buffer and the appropriate test drug After being gassed with a 95:5 mixture of oxygen and carbon dioxide, the flasks were stoppered and incubated in a Dubnoff shaker bath at 37°C for 90 min at 100 cycles per min. Anti-insulin antibody was added to the flasks to prevent the breakdown of insulin according to the method of Malaisse, Malaisse-Lague, and Wright (6).

Isolated islets. Islets were isolated after the method of Lacey and Kostianovsky (7). For experiments in which insulin was to be measured, five islets were transferred to apothecary dram vials containing 0.75 ml of buffer and the appropriate test drug. After being gassed with a 95:5 mixture of oxygen and carbon dioxide, the vials were stoppered with serum stoppers and incubated for 90 min in a Dubnoff shaker bath at 37° C at 100 cycles per min. In these experiments, anti-insulin antibody was not added to the incubation medium.

Oxidation studies. For the oxidation studies, 10 islets were transferred to apothecary dram vials containing 0.5 ml of buffer, a small glass bead, the appropriate test drug, and either glucose-1-14C or glucose-6-14C with a final specific activity of 1.5×10^5 disintegrations per 1 mg of glucose. Each vial was gassed with a mixture of 95:5 oxygen and carbon dioxide and stoppered tightly with a serum stopper from which a polyethylene center well was suspended. Incubations were carried out in a Dubnoff shaker bath for 20 min at 37°C and 150 cycles per min. The reactions were terminated by the immediate injection of 0.2 ml of 4 N H_2SO_4 , and the evolved ${}^{14}CO_2$ was collected at 30°C for 60 min in a Dubnoff shaker bath in 0.1 ml of hydroxide of hyamine (Packard) injected into the center well. All samples were counted in a Packard liquid scintillation counter using automatic external standardization. Results were expressed as counts evolved as ¹⁴CO₂ per 10 islets per 20 min.

"Na accumulation studies. In three separate experiments, 50 islets were transferred to each of two apothecary dram vials containing 0.75 ml of buffer and "Na ranging in specific activity from 10⁷ to 2×10^7 disintegrations per min per 112 μ Eq of sodium. In each experiment, one vial served as a control, and the second served as a test vehicle containing DPH in a concentration of 7.5 μ g/ml. Each vial was gassed with a 95:5 mixture of oxygen and carbon dioxide, stoppered tightly, and incubated in a Dubnoff shaker bath for 90 min at 37°C at 150 cycles per min. At the end of the incubation, each vial was rapidly filtered through a Millipore filter, and the islets were rinsed with 4 ml of buffer. The Millipore filter disc and the adherent islets were transferred with forceps to a Baird Atomic polyethylene counting tube (Baird Atomic, Inc., Bedford, Mass.) and counted in a Nuclear-Chicago gamma counter. The specific activity and total counts in the original incubation medium were determined by counting a 0.5 ml sample of the original 0.75 ml of medium plus 4.0 ml of rinse. Accumulation was then expressed as the fraction of the total ²²Na counts remaining bound per 50 islets.

Insulin assay. Insulin was measured using a single antibody, cellulose column assay developed in our laboratory¹ as a modification of the method of Malaisse et al (6). Standard curves were run with each day's determinations.

Expression of results. All data were compared using the student's t test for comparison of two samples, and 0.05 was chosen as the accepted level of significance.

Sources. Diphenylhydantoin sodium was the gift of Parke, Davis and Co., and the guinea pig antiporcine insulin antiserum was provided by Dr. Peter Wright of the Pharmacology Department, University of Indiana. Porcine insulin for standards in the insulin assay was donated by Eli Lilly and Co. Pentobarbital sodium was purchased from Abbott Laboratories, North Chicago, Ill. Mesantoin was a gift from Sandoz, Inc., Hanover, N. J. Hydroxydiphenylhydantoin was synthesized by Dr. M. Bush of the Pharmacology Department, Vanderbilt University, using the method of Butler (8). Glucose-1-⁴C and glucose-6-⁴⁴C and ²²Na were purchased from New England Nuclear Corp., Boston, Mass. Insulin (¹²⁵I) was purchased from Cambridge Nuclear Corp., Cambridge, Mass. Radioactive ²²Na was purchased from New England Nuclear Corp.

RESULTS

Inhibition of insulin secretion by DPH. Usual therapeutic levels of DPH in man range from 10 μ g/ml plasma for chronic anticonvalsant therapy (9) to 10-20 μ g/ml for acute termination of cardiac arrhythmia (10). The effect of DPH on glucose stimulated insulin secretion by pancreatic pieces is shown in Table I. 5 μ g/ml of DPH did not significantly depress insulin secretion; however, in concentrations of DPH of 7.5, 20, and 40 μ g/ml, insulin secretion was markedly inhibited. Although both of the higher concentrations of DPH were 20% more effective than the 7.5 μ g/ml concentration of DPH, there was no significant difference among the three as determined for a sample size of eight (P >0.05). On the basis of these studies, therefore, a concentration of 7.5 µg/ml of DPH was selected for all further experimentation since it was the lowest concentration which would routinely give a significant inhibition of insulin release.

The effect of pentobarbital on insulin secretion. Because of the possibility that depression of insulin release might be a common property of all anticonvulsants, the effect of pentobarbital on insulin secretion was examined since it was routinely used to anesthetize rats from which isolated iselets were obtained. Insulin secretion was as-

¹ Brendel, K., B. Stocks, and R. Bressler. In preparation.

 TABLE I

 Inhibition of Insulin Secretion by DPH in Pancreas Pieces*

DPH	Insulin secretion mean ±sE	n	Inhibition	Р
µg/ml	μU/100 mg of tissue per 90 min		%	
0	$22,930 \pm 2783$	8		
5.0	$16,792 \pm 1476$	8	25	0.1
7.5	$12,529 \pm 1788$	8	40	< 0.01
20.0	7726 ± 2715	8	64	< 0.005
40.0	8947 ± 1349	8	59	< 0.001

* Glucose concentration in media 200 mg/100 ml.

sayed in pancreatic pieces from rats sacrificed with and without prior anesthesia. One rat was anesthetized by the interperitoneal injection of pentobarbital (6 mg/100 g) prior to decapitation, whereas the second rat was decapitated without prior medication. The data in Table II show that pieces of pancreas from both rats responded identically to 100 and 300 mg/100 ml glucose and to methacholine. Furthermore, the addition of 30 μ g/ml of pentobarbital to the incubations did not inhibit insulin output as compared with unanesthetized controls.

Relationship between anticonvulsant properties of hydantoins and inhibition of insulin secretion. When it became clear that the inhibition of insulin secretion was a specific property of DPH (Table II), the question arose as to whether this inhibitory potential was related to the anticonvulsant properties of DPH or to a peculiar structural component of the DPH molecule. In order to answer this question methylphenylethylhydantoin (mesantoin), also a hydantoin anticonvulsant, and hydroxydiphenylhydantoin, a hydroxylated metabolite of DPH lacking anticonvulsant activity (8), were tested for their ability to inhibit insulin release. The data in Table III show that DPH markedly decreased insulin release as

 TABLE II

 Effect of Pentobarbital Administered In Vitro and In Vivo on Insulin Secretion by Pancreatic Pieces*

TABLE III Comparison of the Effects of DPH, Hydroxy-DPH, and Mesantoin on Insulin Secretion by Pancreatic Pieces*

Experiment	Insulin secretion mean $\pm sE$	n	Р
<u> </u>	µU/100 mg tissue/90 mi	n	••••
Control	$20,658 \pm 1330$	6	
DPH, 7.5 µg/ml	$13,033 \pm 637$	6	< 0.001
Mesantoin, 10 µg/ml	$14,516 \pm 783$	6	< 0.005
OH-DPH, 10 µg/ml	$25,683 \pm 3512$	6	>0.5
OH-DPH, 100 µg/ml	$21,983 \pm 2966$	6	>0.5

* Glucose concentration in media 200 mg/100 ml.

did 10 μ g/ml of mesantoin, whereas hydroxydiphenylhydantoin in concentrations 10 times those of DPH failed to inhibit insulin release, suggesting similar structural requirements for the anticonvulsant and insulin inhibitory properties of the DPH molecule.

Effect of DPH on insulin secretion by pancreatic islets. DPH markedly insulin secretion by isolated pancreatic islets. These data are shown in Table IV.

The effect of DPH on glucose oxidation by pancreatic islets. The data in Table V show that the DPH-induced inhibition of insulin secretion by pancreatic islets is not accompanied by a depression of glucose metabolism. DPH failed to diminish the oxidative conversion of either glucose-1-¹⁴C or glucose-6-¹⁴C to ¹⁴CO₂ by the isolated islets.

The effect of DPH on the action of some insulin secretagogues. Increasing concentrations of glucose result in augmented output of insulin by pancreatic pieces. However, the inhibition of insulin secretion by DPH at 200 mg/100 ml glucose was not overcome by increasing the glucose concentrations of the incubation media. These results are shown in Table VI.

That the inhibition of insulin secretion by DPN is not only resistant to reversal by increased concentrations of glucose but also to reversal by pharmacological insulin secretagogues is evidenced by the failure of methacholine and tolbutamide to reverse the DPH-induced block. These data are shown in Table VII.

	Insulin secretion mean $\pm sE$				
Conditions	Unanesthetized	Anesthetized	P		
	μU/100 mg	tissue/90 min			
Glucose 100 mg/100 ml	7372 + 1200(5)	5978 ±1065(5)	>0.2		
Methacholine $1 \mu g/ml$					
+glucose 100 mg/100 ml	$20,256 \pm 1978(5)$	$21,090 \pm 2755(5)$	>0.5		
Glucose 300 mg/100 ml	$21,472 \pm 1989(5)$	$22,742 \pm 1971(5)$	>0.5		
Pentobarbital 30 µg/ml					
+glucose 100 mg/100 ml	7442 ±2071(5)	_	-		

 TABLE IV

 Inhibition of Insulin Secretion by Isolated

 Pancreatic Islets by DPH*

 DPH	Insulin secretion mean ±sE	n	Р	
µg/ml	μU/5 islets/90 min			
0	3539 ± 250	5		
7.5	516 ± 124	5	<0.001	

* Numbers in brackets indicate the number of incubations carried out. The anesthetized rat received 6 mg/100 g i.p. pentobarbital 20 min before sacrifice.

* Glucose concentration in media 200 mg/100 ml.

1944 J. S. Kizer, M. Vargas-Cordon, K. Brendel, and R. Bressler

TABLE V Effect of DPH on ¹⁴CO₂ Production from Glucose-1-¹⁴C and Glucose-6-14C by Isolated Islets*

	¹⁴ CO ₂ produ	¹⁴ CO ₂ production from:		
DPH	Glucose-1- ¹⁴ C mean ±sE	Glucose-6-14C mean ±SE	n	
g/ml	dpm/5 isle	ets/20 min		
0	1822 ± 40	185 ± 10	4	
7.5	2043 ± 65	245 ± 17	4	

* Each reaction mixture contained 5.5 μ M glucose (1.5 \times 10⁵ dpm glucose-1-14C, 1.5 X 105 dpm glucose-6-14C). Incubations were carried out for 20 min at 37°C. Final reaction volumes were 0.5 ml.

The secretion of insulin by the pancreas is dependent on the presence of extracellular calcium (11). Because of the possibility that DPH might be depressing the activity of the pancreatic β -cell by virtue of a local anesthetic effect similar to that described by Jaanus, Miele, and Rubin for procaine in the adrenal medulla (12), an attempt was made to overcome the DPH inhibition of insulin secretion with increasing concentrations of calcium in the incubation media. The data of Table VIII show that the DPH-induced inhibition of insulin secretion was not reversed by increasing concentrations of calcium in the incubation media.

The effects of ouabain and K^* concentrations on DPH inhibition of insulin secretion. Recent studies on the synaptosome by Festoff and Appel have suggested that the neurophysiological properties of DPH can be attributed to its stimulation of the membrane sodium-potassium-magnesium ATPase and subsequent alteration of resting membrane potential (13).

The possibility that such a mechanism might be operative in the pancreas was assessed by testing the inhibitory activity of DPH in the presence of ouabain (10⁻⁴ mole/liter) and low extracellular K⁺ (0.1 mmole/

TABLE VI Failure of High Concentrations of Glucose to Reverse DPH Inhibition of Insulin Secretion by Pancreas Pieces

TABLE VII Inhibition of Methacholine and Tolbutamide Stimulated Insulin Secretion by Pancreas Pieces by DPH*

DPH	Drug	Insulin secretion mean $\pm se$	n	P‡
µg/ml		µU/100 mg tissue/90 min		
0		$11,977 \pm 2011$	5	
7.5	-	5139 ± 987	5	< 0.01
0	Methacholine, 1 µg/ml	$18,124 \pm 519$	5	< 0.005
7.5	Methacholine, $1 \mu g/ml$	5394 ± 1975	5	
0	Tolbutamide, 250 µg/ml	17,758 ±907	5	<0.005
7.5	Tolbutamide, 250 µg/ml	4950 ± 1015	5	

* Glucose concentration in media 100 mg/100 ml.

‡ As cf. control without any drug.

liter), two conditions which should lead to the inhibition of the ATPase (14).

The data of Table IX show that 0.1 mm K⁺ alone resulted in a 20% stimulation of insulin secretion, which was not, however, significantly different from the controls containing 5 mM K⁺ (P > 0.2), 0.1 mM K⁺ did. however, overcome the DPH-induced inhibition of insulin release and did restore the normal pancreatic insulin response to glucose. Ouabain, on the other hand, was a potent insulin secretagogue (P < 0.001), and likewise, it fully restored the insulin secretory response to control levels.

These observations suggested that DPH might be depressing the excitability of the pancreatic β -cell by virtue of its stimulation of the sodium-potassium-magnesium ATPase and the subsequent reduction of intracellular sodium ion content. This possibility was further examined by determining the net ²²Na uptake in the presence and absence of DPH. As shown in Table X, this experiment was performed on three separate occasions,

TABLE VIII Effect of Calcium on DPH-Induced Inhibition of Insulin Secretion by Pancreas Pieces*

		Insulin secretion							
DPH Glucose	mean ±se	n	P	DPH	Ca++	Insulin secretion mean ±sE	n	Р	
µg/ml	mg/100 ml	μU/100 mg tissue/90 min			µg/ml	mmoles/liter	μU/100 mg		
0	200	21.972 ± 1843	5				tissue/90 min		
Ň	300	32465 ± 2013	5		0	2.0	16,822 ±1959	7	
0	600	$32,100 \pm 1635$	5		7.5	2.0	9003 ±1292	7	< 0.01
0	000	$33,009 \pm 1033$	5	<0.005	7.5	4.0	9272 ± 1331	7	< 0.01
7.5	200	$11,197 \pm 1330$	5	<0.005	75	6.0	0592 1 2019		<0.01
7.5	300	$10,230 \pm 2156$	5	< 0.005	1.5	0.0	9362 ±2016	1	< 0.01
7.5	600	9494 ±675	5	< 0.001					

* Glucose concentration in media 200 mg/100 ml.

 TABLE IX

 Reversal of DPH-Induced Inhibition of Insulin Secretion in

 Pancreas Pieces by Ouabain and Low K⁺ Concentrations*

DPH	Ouabain	K+	Insulin secretion mean $\pm SE$	n	P
µg/ml	moles/liter	mmoles/ liter	μU/100 mg tissue/90 min	<u></u>	
0	0	5.0	$23,259 \pm 1563$	5	_
0	0	0.1	$27,885 \pm 2937$	5	>0.2
0	10-4	5.0	$30,775 \pm 347$	5	< 0.001
7.5	0	5.0	$15,054 \pm 1164$	5	< 0.005
7.5	0	0.1	$26,249 \pm 2284$	5	>0.5
7.5	10-4	5.0	23,716 ± 1540	5	>0.5

* Glucose concentration in media 200 mg/100 ml.

and in each instance, there was a reduction in 22 Na accumulation by the DPH-treated islets of 26–40%.

In a further attempt to delineate the qualitative nature of the DPH-induced inhibition of insulin release, the potassium concentration in the medium bathing the pancreatic pieces was raised to 60 mmoles/liter, a maneuver calculated to antagonize the postulated effect of DPH on the sodium-potassium ATPase and reduce the resting membrane potential of the β -cell, thereby leading to its excitation. As shown in Table XI, 60 mm potassium not only stimulated insulin secretion in the absence of DPH as expected, but also it effectively stimulated insulin secretion in the presence of DPH as well.

DISCUSSION

The data presented in this report demonstrate that therapeutic concentrations of DPH and mesantoin directly inhibit the insulin secretory response to glucose, tolbutamide, and methacholine without a demonstrable alteration in the islets' ability to utilize glucose. Furthermore,

 TABLE X

 Effect of DPH on Accumulation of ³²Na by Isolated Islets

Fraction of total counts accumulation
5.8×10^{-3}
4.8×10^{-3}
$4.68 imes 10^{-3}$
3.38×10^{-3}
3.1×10^{-3}
1.8×10^{-3}

²²Na accumulation in presence and absence of DPH (7.5 μ g/ml) (glucose 200 mg/100 ml).

TABLE XI
Reversal of DPH-Induced Inhibition of Insulin
Secretion of Pancreas Pieces by 60 mM K^{+*}

DPH	К+	Insulin secretion mean $\pm sE$	n	Р
µg/ml	mmoles/ liter	μU/100 mg tissue/90 min		•
0	5.0	$17,275 \pm 1512$	5	
7.5	5.0	9165 ± 415	5	< 0.001
0	60.0	$25,686 \pm 1376$	5	< 0.005
7.5	60.0	$26,746 \pm 1313$	5	< 0.001

* Glucose concentration in media 200 mg/100 ml.

this insulin inhibitory potential is apparently unique to the hydantoins and parallels their anticonvulsant properties. In addition, the capacity of hydantoins to inhibit β -cell excitability is not dependent on a classic local anesthetic effect as suggested by the failure of elevated extracellular calcium ions to stimulate insulin release in the presence of DPH.

The dependence of neuronal excitability on intracellular sodium concentration is well recognized (15-17). Recently, the anticonvulsant properties of DPH have been attributed to its ability to alter intracellular sodium concentration by augmenting the active extrusion of sodium from the intraneuronal space. In the isolated synaptosome, Festoff and Appel observed that the stimulation of the sodium-potassium-magnesium ATPase depends on the ratio of the sodium-potassium ion concentration such that at low ratios of sodium to potassium there is no demonstrable stimulation of the ATPase by DPH. They reasoned that at concentration ratios of intracellular sodium by extracellular potassium of less than 10:1, (conditions obtaining in the normal neuron), DPH would not be expected to appreciably alter resting membrane potential; whereas, higher sodium-potassium ratios might obtain in the injured or rapidly firing neuron a situation wherein DPH should rapidly restore the intracellular sodium concentration to normal and reduce neuronal excitability (13). The reversal of the DPHinduced inhibition of insulin secretion by 0.1 mm potassium and 10^{-4} ouabain and the diminished accumulation of radioactive ²²Na in the presence of DPH suggest that the pancreas DPH may be depressing insulin secretion by virtue of a stimulatory effect on the sodium-potassium ATPase, and that intracellular sodium ion concentration is an important determinant of the secretory response of the islet to glucose, tolbutamide, and methacholine.

It has been observed that DPH has no effect on the excitability of the normal resting neuron (18). However, DPH profoundly depresses the insulin secretion of the β -cell even under normal conditions. Dean and

1946 J. S. Kizer, M. Vargas-Cordon, K. Brendel, and R. Bressler

Matthews measured the resting membrane potential of islets and found it to be approximately -20 mv (19). In the neuron, the resting membrane potential of -90my can be attributed almost entirely to the greater permeability (100-fold) of the neuronal membrane to potassium than to sodium (20). The significantly lower resting membrane potential of the pancreatic β -cell as compared with the neuron could result from either a decreased potassium permeability or an increased sodium permeability of the membrane, assuming these ionic species to be the major determinants of the resting membrane potential. If the lower resting potential of the pancreatic β -cell were, in fact, partially the result of an increase in the membrane sodium permeability, then it is conceivable that islet sodium influx would be larger than that of the neuron. Such a rise in intracellular sodium might result in an intracellular sodium to extracellular potassium concentration ratio significantly greater than 10:1 at the membrane, and DPH would, therefore, be expected to stimulate the sodium-potassiummagnesium ATPase of the β -cell even under normal conditions, since there is apparently a constant baseline electrical activity of the islet at all times (19).

Such an interpretation of our data is also supported by the observation that 60mm potassium not only reverses the DPH-induced inhibition of insulin secretion but also stimulates insulin secretion in the presence of DPH. It appears likely that elevation of the extracellular potassium ion concentration reduces the sodiumpotassium ratio to approximately 1:1, well within the range of the sodium-potassium concentration ratios wherein Festoff and Appel demonstrated no stimulation of the sodium-potassium ATPase by DPH (13). 60 mm potassium, therefore, would essentially neutralize the depression of β -cell excitability by DPH and in addition, because of its reduction of transmembrane potential, would result in just as great a stimulation of insulin secretion in the presence of DPH as in its absence.

A number of criticisms can be raised to such an interpretation of our data. First, it may be argued that DPH might block the passive flux of sodium since accumulation of "Na is dependent upon passive as well as active transport. If the blocking of the passive diffusion of sodium were an important determinant of its action, however, ouabain, 0.1 mm potassium, and 60 mm potassium would not be expected to reverse the inhibition of insulin release by DPH since they have no known direct effect on passive sodium diffusion. Secondly, it could be argued that DPH increases intracellular water such that the effective concentration of sodium within the cell is subliminal. An increase of intracellular water, however, could not give rise to the observed increase in "Na accumulation, although an increase in intracellular water might well be observed as a result of a reduced intracellular sodium ion concentration. Thirdly, one could argue that we have not demonstrated that the intracellular concentration of sodium is a determinant of the insulin secretory response of the β -cell to glucose, even assuming that DPH were to accelerate the sodium-potassium-magnesium ATPase since it is possible that ATP is a ratelimiting substrate for the process of insulin secretion. If ATP were being drawn away from other cellular processes by the DPH accelerated ATPase, a decrease in the utilization of glucose might be expected because of the necessary phosphorylation of glucose prior to its further oxidation. From the glucose utilization data, however, it is apparent that CO₂ production from glucose was not diminished.

Admittedly, such evidence implicating the sodium-potassium ATPase as the main locus of the insulin inhibitory effects of DPH is largely indirect, and further studies utilizing rates of ²²Na efflux will be necessary to determine the validity of such an interpretation of the data.

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DPH Inhibition of Insulin Secretion 1947

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