# Ionic Basis of Phenytoin Sodium Inhibition of Insulin Secretion in Pancreatic Islets

CAROLINE S. PACE AND ELIZABETH LIVINGSTON

**SUMMARY**

**The possibility that phenytoin sodium has a dual role in blocking Ca and Na channels in the pancreatic β-cell membrane was studied to clarify the mechanism of action of the drug in inhibiting insulin release. Glucose and veratridine were used to activate the Ca and Na channels, respectively. The increase in insulin release induced by 16.7 mM glucose or 200 μM veratridine was inhibited 77% and 60%, respectively, by 100 μM phenytoin, whereas the increase in the rate of glycolysis was inhibited 74% and 100%, respectively. An increase in extracellular Ca from 2.5 to 5.0 mM attenuated the inhibitory effect of phenytoin on both the metabolic and secretory responses to glucose and veratridine. This Ca-dependent reversal was greater for glucose than for veratridine. Furthermore, 1.0 mM ouabain partially reversed the inhibitory effect of phenytoin on the secretory responses to both stimulants. This may be attributed to the indirect effect of ouabain in increasing intracellular Ca. However, ouabain together with phenytoin almost completely blocked glycolytic flux measured in the presence of either glucose or veratridine. These results suggest that phenytoin may block the stimulation of passive Na and/or Ca transport in the β-cell membrane.**

**DIABETES *28*:1077–1082, December 1979.**

The glucose intolerance following the administration of phenytoin sodium has been attributed to a direct inhibitory action of the drug on glucose-induced insulin secretion.[1–3] Although its precise mode of action is not known, the available evidence supports the concept that phenytoin stimulates the Na-K pump in the islet cell membrane.[2] This is based on the observations that low extracellular K or ouabain reverses the effects of phenytoin. Studies on the inhibitory effect of phenytoin on neurons and synaptosomes indicated that the drug may inhibit passive Na[4–6] or Ca influx.[7,8] In pancreatic β-cells, activation of Na or Ca channels by veratridine[9,10] or glucose,[11] respectively, induces insulin secretion. However, tetrodotoxin (TTX), a specific blocker of the Na channel in excitable membranes,[12] inhibits veratridine-induced electrical activity,[13] glycolytic flux,[10,13] and insulin secretion,[9,10,13,15] but does not alter the effects of glucose.[13–15]

The effects of phenytoin on veratridine and glucose-induced metabolic and secretory responses were examined to determine if the drug has a dual role in blocking both Na and Ca entry sites in the pancreatic β-cell membrane.

## METHODS AND PROCEDURES

Islets of Langerhans were isolated by the collagenase technique from male Sprague Dawley rats (250–300 g) fed ad libitum.[16] For the secretory studies, 15–25 islets were placed in a tube containing 1.0 ml of a medium of the following ionic composition (mM): $Na^+$ 143.3, $K^+$ 5.0, $Ca^+$ 2.5, $Mg^{2+}$ 1.2, $Cl^-$ 123.5, $HCO_3^-$ 24.6, $H_2PO_4^-$ 1.2, and $SO_4^{2-}$ 1.2. The medium was supplemented with 0.3% (w/v) bovine serum albumin. The tubes were capped with rubber stoppers and maintained at 37°C in a metabolic shaker (100 strokes/min) and at pH 7.4 by gassing with 95% $O_2$ and 5% $CO_2$. Islets were preincubated in medium containing 2.8 mM glucose for 30 min, after which the medium was withdrawn and fresh medium containing the desired agents was added. The islets were incubated for an additional 60 min, after which the medium was withdrawn and stored at −20°C until assayed for insulin content.[17]

For the metabolic studies 20–25 islets were placed into 7 × 35-mm tubes containing 25 μl of the above medium with the addition of 2.8 mM glucose. Each tube was then placed in a scintillation vial, stoppered, and preincubated for 30 min at 37°C in an atmosphere of 95% $O_2$ and 5% $CO_2$. Medium (25 μl) containing [5-$^3$H] glucose (0.4–2.0 mCi/mmol) and the indicated agents was then added to each tube to achieve the desired final concentration. After 60 min of incubation, metabolism was terminated by the ad-

From the Department of Physiology and Biophysics, University of Alabama Medical School, Birmingham, Alabama 35294.
Received for publication 11 April and in revised form 2 August 1979.



Downloaded from http://diabetesjournals.org/diabetes/article-pdf/28/12/1077/350103/28-12-1077.pdf by guest on 23 June 2024

TABLE 1
Effect of phenytoin on insulin secretion due to glucose or veratridine

| Experiment | Glucose (mM) | Veratridine ($\mu$M) | Phenytoin ($\mu$M) | Insulin release ($\mu$U/islet/h) Mean ± SE (N) | P value Test vs. control |
|---|---|---|---|---|---|
| I. A | 5.6 | — | — | 13.6 ± 0.4 (20) | |
| B | 5.6 | — | 100 | 10.2 ± 1.5 (8) | NS* (B,A) |
| C | 16.7 | — | — | 46.3 ± 3.0 (20) | <0.001 (C,A) |
| D | 16.7 | — | 100 | 21.2 ± 1.4 (20) | <0.001 (D,C) (D,B) |
| II. A | 5.6 | — | — | 11.1 ± 1.2 (20) | |
| B | 5.6 | — | 100 | 12.2 ± 2.0 (8) | NS (B,A) |
| C | 5.6 | 200 | — | 40.9 ± 2.6 (20) | <0.001 (C,A) |
| D | 5.6 | 200 | 100 | 23.0 ± 2.2 (18) | <0.001 (D,C) (D,B) |

Islets were batch incubated for a preincubation period of 30 min in a bicarbonate-buffered salt solution containing 2.8 mM glucose with an atmosphere of 95% $O_2$ and 5% $CO_2$ at 37°C. The islets were then incubated for 60 min in the presence of the indicated agents. The medium withdrawn was stored at −20°C until assayed for insulin content.
* NS = not significant.

dition of 50 $\mu$l of 0.5 N HCl. Controls consisting of tubes containing radioactive medium without islets and also $^3H_2O$ standards were treated in the same way. Glucose utilization was determined by the rate of $^3H_2O$ formed from [5-$^3$H] glucose metabolized by the islet cells using liquid scintillation spectrometry.[18] The results were corrected for the blanks and the recovery of $^3H_2O$. Phenytoin was obtained from Sigma Chemical Company, St. Louis, Missouri.

**RESULTS**

The secretory response to 16.6 mM glucose or 200 $\mu$M veratridine was inhibited 77% and 60%, respectively, by 100 $\mu$M phenytoin (Table 1). In comparison, the increase in glycolytic flux over basal values due to either glucose or veratridine was partially (74%) or completely blocked, respectively, by 100 $\mu$M phenytoin (Table 2).

To test the possibility that phenytoin interferes with Ca influx, we determined the effect of increasing the level of Ca in the extracellular medium on the inhibitory effect of phenytoin on both the secretory and metabolic responses to glucose and veratridine. In our initial studies, we determined the dose-response effect of phenytoin on insulin release (Figure 1). At a level of 200 $\mu$M, phenytoin completely blocked the secretory response to glucose (Figure 1) and veratridine (data not shown) to basal levels of insulin release. We chose a concentration of 100 $\mu$M for further studies, since this level produced a consistent and significant inhibition of insulin release. Although increasing $[Ca]_0$ produced a small increase in the secretory response to 16.7 mM glucose alone, it is clear that Ca also attenuated the inhibitory effect of phenytoin (Figure 1). For example, with 16.7 mM glucose, an increase in $[Ca]_0$ from 2.5 to 5.0 mM doubled release in the presence of 100 or 200 $\mu$M phenytoin, but only increased insulin release by 25% in its absence. The increase in $[Ca]_0$ did not influence basal levels of insulin release obtained in the presence of 5.6 mM glucose (Table 3).

A twofold increase in $[Ca]_0$ did not affect the basal or stimulated rates of glucose utilization. However, the increase in $[Ca]_0$ reversed the inhibitory effect of 100 $\mu$M phenytoin on glycolysis from about 20% to 65% of the control value obtained with glucose in the absence of phenytoin (Table 4).

An increase in $[Ca]_0$ also reversed the inhibitory effect of 100 $\mu$M phenytoin on veratridine-induced insulin release without significantly increasing the secretory response to veratridine alone (Table 3). However, this effect was small in

TABLE 2
Effect of phenytoin on glucose utilization due to glucose or veratridine

| Experiment | Glucose (mM) | Veratridine ($\mu$M) | Phenytoin ($\mu$M) | Glycolytic flux (pmol/islet/h) Mean ± SE (N) | P value Test vs. control |
|---|---|---|---|---|---|
| I. A | 5.6 | — | — | 14.2 ± 0.9 (8) | |
| B | 5.6 | — | 100 | 15.1 ± 1.2 (8) | NS*(B,A) |
| C | 16.7 | — | — | 75.5 ± 6.6 (8) | <0.001 (C,A) |
| D | 16.7 | — | 100 | 30.1 ± 3.2 (12) | <0.005 (D,B) <0.001 (D,C) |
| II. A | 5.6 | — | — | 13.3 ± 0.8 (8) | |
| B | 5.6 | — | 100 | 16.6 ± 1.1 (8) | NS (B,A) |
| C | 5.6 | 200 | — | 50.0 ± 4.0 (8) | <0.001 (C,A) |
| D | 5.6 | 200 | 100 | 12.8 ± 2.8 (8) | NS (D,B) <0.001 (D,C) |

Islets were batch incubated in 2.8 mM glucose for 30 min, after which additional medium containing [5-$^3$H] glucose and the indicated agents was added to achieve the desired final concentration. Metabolism was terminated after 60 min by adding HCl. Glucose utilization was determined by the rate of $^3H_2O$ formed using liquid scintillation spectrometry.
* NS = not significant.



Downloaded from http://diabetesjournals.org/diabetes/article-pdf/28/12/1077/350103/28-12-1077.pdf by guest on 23 June 2024

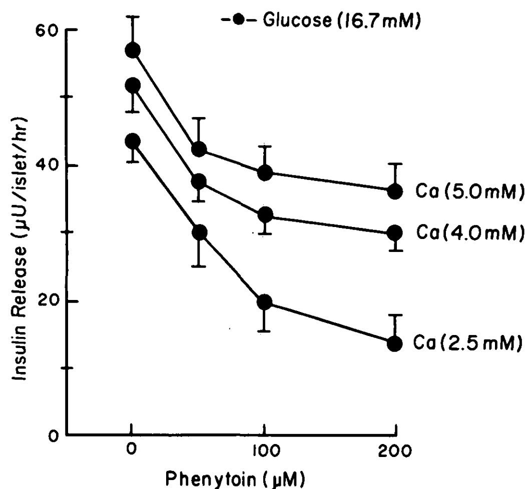


**FIGURE 1. The effect of increasing $[Ca]_0$ on the phenytoin dose-dependent inhibitory effect on glucose-induced insulin secretion. (See footnote to Table 1 for experimental details.) Each point represents the mean ± SE of at least 12 observations. An increase in $[Ca]_0$ did not significantly change the basal release of insulin in the presence of 5.6 mM glucose. The value of insulin release in the presence of 200 μM phenytoin and 2.5 mM Ca is not different from the basal value obtained with 5.6 mM glucose.**

that phenytoin inhibited the veratridine-induced increase in insulin release by 60% in the presence of 2.5 mM Ca and by 50% in the presence of 5.0 mM Ca as compared with 80% and 40%, respectively, with glucose. Although 100 μM phenytoin completely blocked the stimulation of glycolytic flux due to veratridine, a twofold increase in $[Ca]_0$ reversed the inhibition. The increase in $[Ca]_0$ did not affect the values of glucose utilization obtained in the absence or presence of veratridine.

We have previously shown that the increase in glycolysis due to veratridine is blocked by 1.0 mM ouabain or 3.0 μM TTX.[10,13] This supported our hypothesis that the increase in intracellular Na due to veratridine serves as a stimulant of glucose utilization via activation of an ATP-dependent Na-K pump. Ouabain also reduced the increase in glycolytic flux due to glucose.[10,13,19] The observation by other investigators that ouabain counteracts the inhibitory influence of phenytoin on glucose-induced insulin release led to the suggestion that phenytoin activates the Na-K pump.[2] According to this hypothesis, one would expect phenytoin to stimulate or, at least, not to inhibit (as reported in the present studies) the effect of veratridine and glucose on glucose utilization. We examined ouabain's effect on the inhibitory influence of phenytoin on the metabolic and secretory effects of veratridine and glucose. As found previously, 1.0 mM ouabain does not affect basal insulin release or secretory responses to high glucose or veratridine[10,13] (Table 3). In the present studies, 1.0 mM ouabain partially reversed the effect of 100 μM phenytoin on the secretory responses (Table 5), but not the metabolic responses (Table 6), due to 16.7 mM glucose or 200 μM veratridine. In fact, the combination of ouabain and phenytoin reduced glycolytic flux substantially below the basal values obtained with 5.6 mM glucose alone (Table 2) or together with either ouabain or phenytoin (Tables 2 and 6).

## DISCUSSION

The results of our studies are consistent with the view that phenytoin inhibits the permeability of the stimulated β-cell membrane to Na and Ca. It was shown previously that 3.0 μM TTX effectively blocked veratridine-induced insulin release but had little or no effect on release due to glucose.[9,10,15] It may be inferred from our results that phenytoin affects TTX-sensitive Na channels in addition to another site, which may be the glucose-activated Ca channel. It has been well documented that phenytoin inhibits Na conductance in axons.[4–6] In our studies phenytoin at a level of 200 μM blocked the increase in insulin release and glycolytic flux due to veratridine plus basal glucose to that obtained with basal glucose alone. Under the same conditions, 100 μM phenytoin partially reduced insulin release by 74% and blocked glycolysis to basal levels. These results are incompatible with the theory that phenytoin inhibits insulin release by activating an energy-dependent Na-K pump. The reduction in glycolysis may be due to phenytoin-induced reduction of Na influx, thereby reducing the demand on the Na-K pump.[10,13]

Phenytoin also inhibits Ca uptake by K-depolarized synaptosomes[7] or brain slices,[8] suggesting that the drug also blocks Ca channels. Interestingly, TTX had no effect on Ca uptake in brain slices associated with stimulation of norepinephrine release.[8] Increasing the $[Ca]_0$ attenuated the inhibitory effect of phenytoin on release of insulin, as was also found with release of norepinephrine from brain slices.[8] We have also demonstrated the antagonistic action of Ca on the inhibitory effect of phenytoin on insulin release and glucose utilization. Glucose stimulation of glycolysis has been shown to be reduced by the absence of $Ca_0$,[20,21] whereas

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/28/12/1077/350103/28-12-1077.pdf by guest on 23 June 2024

TABLE 3
Effect of increased $[Ca]_0$ on phenytoin inhibition of veratridine-induced metabolic and secretory responses

| Experiment | Glucose | Veratridine | Ca | Phenytoin | Insulin release (μU/islet/h) Mean ± SE (N) | P value | Glycolytic flux (pmol/islet/h) Mean ± SE (N) | P value |
|---|---|---|---|---|---|---|---|---|
| A | 5.6 | — | 2.5 | — | 10.4 ± 3.1 (12) | | 15.2 ± 0.8 (12) | |
| B | 5.6 | — | 5.0 | — | 13.2 ± 3.2 (12) | NS* (B,A) | 18.3 ± 1.6 (12) | NS (B,A) |
| C | 5.6 | 200 | 2.5 | — | 38.4 ± 4.6 (12) | <0.001 (C,A) | 53.4 ± 3.5 (12) | <0.001 (C,A) |
| D | 5.6 | 200 | 2.5 | 100 | 24.0 ± 2.0 (12) | <0.02 (D,C)<br><0.005 (D,A) | 13.2 ± 3.1 (12) | NS (D,A)<br><0.001 (D,C) |
| E | 5.6 | 200 | 5.0 | — | 49.2 ± 4.2 (12) | NS (E,C) | 57.3 ± 4.2 (12) | NS (E,C) |
| F | 5.6 | 200 | 5.0 | 100 | 34.3 ± 2.6 (12) | NS (F,C)<br><0.01 (F,E) | 28.2 ± 3.8 (12) | <0.001 (F,E)<br><0.05 (F,B) |

See footnote to Tables 1 and 2.
* NS = not significant.

TABLE 4
Effect of increased $[Ca]_0$ on phenytoin inhibition of glucose utilization

| Experiment | Glucose (mM) | Ca (mM) | Phenytoin (μM) | Glycolytic flux (pmol/islet/h) Mean ± SE (N) | P value |
|---|---|---|---|---|---|
| A | 5.6 | 2.5 | — | 16.1 ± 2.1 (12) | |
| B | 5.6 | 5.0 | — | 18.2 ± 1.2 (12) | NS* (B,A) |
| C | 16.7 | 2.5 | — | 80.8 ± 7.3 (12) | <0.001 (C,A) |
| D | 16.7 | 2.5 | 100 | 28.3 ± 3.1 (12) | <0.01 (D,A)<br><0.001 (D,C) |
| E | 16.7 | 5.0 | — | 75.4 ± 6.8 (12) | NS (E,C) |
| F | 16.7 | 5.0 | 100 | 55.3 ± 4.4 (12) | <0.02 (F,E)<br><0.001 (F,D) |

See footnote to Table 2.
* NS = not significant.

insulin secretion is almost completely inhibited.[21] An impressive correlation (r = 0.964) exists between lactate output, used as an indicator of glycolytic flux, and the net uptake of $^{45}Ca$ due to increasing levels of glucose.[22] An increase of extracellular Ca over a range of 0–2.0 mM is also associated with an increase in the net uptake of $^{45}Ca$ and insulin release induced by 16.7 mM glucose.[21] Data are not available demonstrating the effect of increasing Ca over a range of 2.5–5.0 mM on glycolytic flux associated with the net uptake of $^{45}Ca$. However, we have found that an increase of Ca from 2.5 to 5.0 mM did not affect glucose-induced glycolytic flux but increased insulin release by 25%. Nevertheless, in considering the above information, it appears that a close link exists between the uptake of Ca and both glycolytic flux and insulin release at levels of Ca below 2.0 mM when determined in the presence of 16.7 mM glucose. This information, together with our demonstration of Ca-induced reversal of phenytoin inhibition of glycolysis and insulin release, supports the view that phenytoin inhibits Ca uptake via the glucose-activated Ca channel.

An increase in $[Ca]_0$ led to a smaller reversal of the inhibitory influence of phenytoin on veratridine-induced insulin release, but substantially increased the rate of glycolytic flux. The secretory response to veratridine has been observed to be associated with an increase in the uptake of $^{45}Ca$.[9] We have found, in addition, that the absence of $[Ca]_0$ or the addition of Co antagonizes the secretory and metabolic responses to veratridine.[13] Apparently, the veratridine-evoked increase in Na permeability, leading to an increase in $Na_i$ and depolarization, may increase the cytosolic level of Ca by stimulating Na-Ca countertransport[23] or by opening voltage-dependent Ca channels.[9,13] Intracellular Na may also increase cytosolic Ca by mobilization from intracellular-bound pools.[9] Possibly, a twofold increase in extracellular Ca in the presence of veratridine and phenytoin leads to accumulation of intracellular Ca that otherwise may not occur as a consequence of an increase in intracellular Na.

Although phenytoin, at a concentration 25% of that used in the present studies, was found to reduce net $^{22}Na$ uptake in islet cells by 26%–40%,[2] this may be indicative of the action of phenytoin on passive as well as active transport of Na. Evidence that phenytoin affects passive Na transport was obtained by the observation that it did not stimulate the active uptake of Rb[24] (Rb was used as a substitute for K in

TABLE 5
Effect of ouabain on inhibitory influence of phenytoin on secretory responses to veratridine or glucose

| Experiment | Glucose (mM) | Veratridine (μM) | Phenytoin (μM) | Ouabain (mM) | Insulin release (μU/islet/h) Mean ± SE (N) | P value Test vs. control |
|---|---|---|---|---|---|---|
| I. A | 5.6 | — | — | 1.0 | 10.3 ± 2.1 (10)* | |
| B | 16.7 | — | — | — | 53.2 ± 6.2 (10) | <0.001 (B,A) |
| C | 16.7 | — | 100 | — | 26.8 ± 3.4 (10) | <0.005 (C,B)<br><0.025 (C,A) |
| D | 16.7 | — | — | 1.0 | 58.2 ± 5.3 (10) | NS† (D,B)<br><0.001 (D,C) |
| E | 16.7 | — | 100 | 1.0 | 42.1 ± 3.2 (10) | <0.01 (E,C)<br><0.001 (E,D) |
| II. A | 5.6 | — | — | 1.0 | 12.3 ± 2.4 (10)* | |
| B | 5.6 | 200 | — | — | 45.2 ± 2.2 (10) | <0.001 (B,A) |
| C | 5.6 | 200 | 100 | — | 28.0 ± 1.9 (10) | <0.001 (C,B)<br><0.001 (C,A) |
| D | 5.6 | 200 | — | 1.0 | 47.4 ± 3.5 (10) | NS (D,B)<br><0.001 (D,C) |
| E | 5.6 | 200 | 100 | 1.0 | 35.2 ± 2.0 (10) | <0.025 (E,C)<br><0.01 (E,D) |

See footnote to Table 1.
* Value obtained is not significantly different from value obtained with 5.6 mM glucose without ouabain or with 5.6 mM glucose plus phenytoin (see Table 1).
† NS = not significant.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/28/12/1077/350103/28-12-1077.pdf by guest on 23 June 2024

TABLE 6
Effect of ouabain on inhibitory influence of phenytoin on metabolic responses to veratridine or glucose

| Experiment | Glucose (mM) | Veratridine (μM) | Phenytoin (μM) | Ouabain (mM) | Glycolytic flux (pmol/islet/h) Mean ± SE (N) | P value Test vs. control |
|---|---|---|---|---|---|---|
| I. A | 5.6 | — | — | 1.0 | 12.9 ± 1.2 (6)* | |
| B | 16.7 | — | — | — | 80.2 ± 3.1 (6) | <0.001 (B,A) |
| C | 16.7 | — | 100 | — | 24.5 ± 2.6 (8) | <0.002 (C,A) |
| D | 16.7 | — | — | 1.0 | 38.3 ± 3.2 (6) | <0.001 (D,A) (D,B) |
| E | 16.7 | — | 100 | 1.0 | 2.3 ± 1.5 (6) | <0.003 (E,A) (E,C) |
| II. A | 5.6 | — | — | 1.0 | 16.4 ± 2.1 (6)* | |
| B | 5.6 | 200 | — | — | 56.2 ± 4.1 (6) | <0.001 (B,A) |
| C | 5.6 | 200 | 100 | — | 16.1 ± 3.2 (6) | NS* (C,A) <0.001 (C,B) |
| D | 5.6 | 200 | — | 1.0 | 19.4 ± 2.4 (6) | NS (D,A) <0.001 (D,B) |
| E | 5.6 | 200 | 100 | 1.0 | 1.0 ± 0.5 (6) | <0.001 (E,A) <0.005 (E,C) |

See footnote to Table 2.
* Value obtained is not significantly different from value obtained with 5.6 mM glucose without ouabain or with 5.6 mM glucose plus phenytoin (see Table 2).
† NS = not significant.

examining Na-K pump activity). However, conditions known to inhibit the activity of the pump, namely, ouabain and low extracellular K, reversed the inhibition of insulin release by phenytoin.[2] These results were interpreted as being indirect evidence that phenytoin stimulates active Na transport. However, inhibition of the Na-K pump leads to accumulation of intracellular Na. This in turn may lead to an increase in intracellular Ca by Na-Ca countertransport.[23] It is theoretically possible that ouabain increased insulin release in the presence of phenytoin via this mechanism regardless of the effect of phenytoin on passive or active Na transport. In the present study, ouabain partially reversed the effect of phenytoin on the secretory but not the metabolic responses due to high glucose or veratridine. The abolishment of glycolytic flux in the presence of ouabain and phenytoin indicates that phenytoin does not increase active Na efflux, but may instead decrease passive Na influx in addition to passive Ca influx. Consequently, phenytoin may be inhibiting an energy-dependent process insensitive to ouabain and/or to the level of ouabain used in these studies. Matschinsky and Ellerman[19] found 1.0 and 5.0 mM ouabain to reduce glucose-induced lactate formation by 50% and 87%, respectively. The differential effect of phenytoin plus ouabain on the metabolic and secretory responses to glucose and veratridine is another example of the dissociation between the metabolic and secretory responses of islet cells due to manipulation of ionic gradients.[13]

Phenytoin also hyperpolarizes the β-cell membrane and inhibits glucose-induced spike activity.[25] This observation has also been interpreted as indicative of the stimulatory effect of this drug on the Na-K pump. However, hyperpolarization of the β-cell may occur not only as a result of increased intracellular K brought about by increased pump activity, but also by a decrease in Na conductance. Exposure of the squid axon to phenytoin also leads to hyperpolarization, but this event occurs even in the presence of ouabain,[6] eliminating the possibility of the influence of an electrogenic Na-K pump on phenytoin-induced membrane hyperpolarization.

It appears that the inhibitory action of phenytoin on the pancreatic β-cell can be ascribed to the blockade of Na or Ca channels, depending on the specificity of the given stimulant. Studies of the direct actions of phenytoin on $^{22}$Na, $^{42}$K, and $^{45}$Ca fluxes are necessary to further confirm the ionic mechanism of this drug's action.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the skilled technical assistance of Becky Swindall. This work was supported by grants from the Juvenile Diabetes Foundation, the Kroc Foundation, the Diabetes Trust Fund, and NIH (AM21973). The guinea pig insulin standard used in the radioimmunoassay of insulin was a gift from Eli Lilly and Company. C.S.P. is a recipient of a Research Career Development Award (AM00429).

## REFERENCES

[1] Levin, S. R., Booker, J., Smith, D. F., and Grodsky, G. M.: Inhibition of insulin secretion in vitro by diphenylhydantoin. J. Clin. Endocrinol. Metab. *30*:400-01, 1970.

[2] Kizer, S. J., Vargas-Cordon, M., Brendel, K., and Bressler, R.: The in vitro inhibition of insulin secretion by diphenylhydantoin. J. Clin. Invest. *49*:1942-48, 1970.

[3] Levin, S. R., Grodsky, G. M., Hagura, R., and Smith, D.: Comparison of the inhibitory effects of diphenylhydantoin and diazoxide upon insulin secretion from the isolated perfused pancreas. Diabetes *21*:856-62, 1972.

[4] Pincus, J. H.: Diphenylhydantoin and ion flux in lobster nerves. Arch. Neurol. *26*:4-10, 1972.

[5] Lipicky, R. J., Gilbert, D. L., and Stillman, I. M.: Diphenylhydantoin inhibition of sodium conductance in squid giant axon. Proc. Natl. Acad. Sci. USA *69*:1758-60, 1972.

[6] Perry, J. G., McKinney, L., and DeWeer, P.: The cellular mode of action of the antiepileptic drug, 5,5'-diphenylthydantoin Nature *272*:271-73. 1978.

[7] Sohn, P. S., and Ferrendelli, J. A.: Inhibition of Ca++ transport into rat brain synaptosomes by diphenylhydantoin. J. Pharm. Exp. Ther. *185*:272-75, 1973.

[8] Pincus, J. H., and Lee, S. H.: Diphenylhydantoin and calcium. Arch. Neurol. *29*:239-44, 1973.

[9] Donatsch, P., Lowe, D. A., Richardson, R. P., and Taylor, P.: The functional significance of sodium channels in pancreatic beta-cell membranes. J. Physiol. (Lond.) *267*:357-76, 1977.

[10] Pace, C. S., and Blaustein, M. P.: Effects of neurotoxins on pancreatic islets: elucidation of a possible role for sodium channels in the secretory response. Biochim. Biophys. Acta *585*:100-06, 1979.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/28/12/1077/350103/28-12-1077.pdf by guest on 23 June 2024

[11] Malaisse, W. J., Herchuelz, G., Somers, G., Boschero, A. C., Hutton, J. C., Kawazu, S., Sener, A., Atwater, I. J., Duncan, G., Ribalet, B., and Rojas, E.: Regulation of calcium fluxes and their regulatory roles in pancreatic islets. *In* Calcium Transport and Cell Function. New York, The New York Academy of Sciences, 1978, pp. 562–82.

[12] Ulbricht, W.: The effect of veratridine on excitable membranes of nerve and muscle. Ergeb. Physiol. *61*:18–71, 1969.

[13] Pace, C. S.: Activation of Na channels in islet cells: metabolic and secretory effects. Am. J. Physiol. *237*(2):E130–E135, 1979.

[14] Meissner, H. P., and Schmelz, H.: Membrane potential of β-cells in pancreatic islets. Pflugers Arch. *351*:195–206, 1974.

[15] Milner, R. D. G., and Hales, C. N.: The interaction of various inhibitors and stimuli of insulin release studied with rabbit pancreas in vitro. Biochem. J. *113*:473–79, 1969.

[16] Lacy, P., and Kostianovsky, M.: Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes *16*:35–39, 1967.

[17] Wright, P. H., Makulu, D. R., Vichick, D., and Sussman, K. E.: Insulin immunoassay by back-titration: some characteristics of the technique and the insulin precipitant action of alcohol. Diabetes *20*:33–45, 1971.

[18] Ashcroft, S. J. H., Weersinghe, L. C. C., Bassett, J. M., and Randle, P. J.: The pentose cycle and insulin release in mouse pancreatic islets. Biochem. J. *126*:525–32, 1972.

[19] Matschinsky, F. M., and Ellerman, J.: Dissociation of the insulin releasing and metabolic functions of hexoses in islets of Langerhans. Biochem. Biophys. Res. Commun. *50*:193–99, 1973.

[20] Hellman, B., Idahl, 1.-A. Sehlin, J., and Täljedal, I.-B.: The pancreatic β-cell recognition of insulin secretagogues: effects of calcium and sodium on glucose metabolism and insulin release. Biochem. J. *138*:33–45, 1974.

[21] Malaisse, W. J., Hutton, J. C., Sener, A., Levy, J., Herchuelz, A., Devis, G., and Somers, G.: Calcium antagonists and islet function: VII. Effect of calcium deprivation. J. Membr. Biol. *38*:193–208, 1978.

[22] Sener, A., Levy, J., and Malaisse, W. J.: The stimulus-secretion coupling of glucose induced insulin release: does glycolysis control calcium transport of the β-cell? Biochem. J. *156*:521–25, 1976.

[23] Baker, P. F., Blaustein, M. P., Hodgkin, A. L., and Steinhardt, R. A.: The influence of calcium on sodium efflux in squid axons. J. Physiol. (Lond.) *200*:431–58, 1969.

[24] Sehlin, J., and Täljedal, I.-B.: Transport of rubidium and sodium in pancreatic islets. J. Physiol. (Lond.) *242*:505–15, 1974.

[25] Matthews, E. K., and Sakamoto, Y.: Pancreatic islet cells: electrogenic and electrodiffusional control of membrane potential. J. Physiol. (Lond.) *246*:439–57, 1975.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/28/12/1077/350103/28-12-1077.pdf by guest on 23 June 2024