Effect of Local Anesthetics on Insulin Secretion by Pancreas Pieces

R. Bressler, M.D., and K. Brendel, Ph.D., Tucson

SUMMARY

Because in vitro secretion is calcium dependent, a study was carried out to ascertain the effect of local anesthetics (LA) on glucose-induced insulin secretion by pancreas pieces.

Different classes of LA affected insulin secretion differently. Nonantiacetylcholine type LA with uncharged amino groups (holocaine, propranolol) depressed glucose-induced insulin secretion progressively as their concentrations were raised (10^{-3} to 1 mM). The inhibition was more marked at lower compared to higher calcium concentrations.

The antiacetylcholine type LA (mepivicaine, lidocaine) with charged tertiary amino groups had complex effects on insulin secretion. At low concentrations (10^{-4} mM) they acted as anticholinergic agents in their inhibitions of Mecholyl-stimulated insulin secretion. Complete blockade of Mecholyl-stimulated secretion was found at calcium concentrations of 0.5 and 3.0 mM. This concentration of LA did not, however, depress glucose-induced insulin secretion. At an LA concentration of 10^{-3} mM inhibition of glucose-stimulated insulin secretion occurred, which was not reversed by higher concentrations of calcium. At still higher LA concentrations (0.05 to 1.0 mM) the inhibitory effect on glucose-induced insulin secretion diminished, disappeared and became a stimulatory effect, which was abolished by the addition of atropine. DIABETES *20*:721-28, November, 1971.

The β cells of the pancreatic islets of Langerhans are known to be innervated by fibers from both the parasympathetic and sympathetic systems.[1-3] It has been ascertained that adrenergic drugs have marked effects on insulin secretion both in vitro and in vivo. Beta adrenergic stimulation (N-isopropylnorepinephrine) enhances, whereas α adrenergic stimulation (epinephrine, norepinephrine) inhibits insulin secretion.[4-7] It has also been found that in vivo and in vitro cholinergic stimulation via either parasympathetic drugs or vagal stimulation has an insulin stimulatory effect, which is blocked by the simultaneous use of atropine.[8-11]

A number of studies have been concerned with the role of various extracellular cations in in vitro insulin secretion. These investigations have shown that insulin secretion is dependent upon the presence of sodium and calcium in the incubation media.[12,13]

Studies on the role of extracellular calcium in catecholamine release from the adrenal medulla have shown that propranolol, considered to be a classic β adrenergic blocking agent, was capable of inhibiting the stimulatory action of calcium.[14,15] It was concluded that propranolol was acting as a local anesthetic to inhibit calcium fluxes which depressed catecholamine secretion.[14]

In recent communications from this laboratory, data were presented which showed that aryl substituted secondary aminoethanols such as 1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol (propranolol) and 4-(2-isopropylamino-1-hydroxyethyl) methanesulfonanilide (Sotalol) inhibited in vivo insulin release in response to a number of insulin secretagogues.[16,17] It was postulated that these agents were acting as local anesthetics which decreased the calcium permeability of the pancreatic β cells.[16,18] Because extracellular calcium appears to be a key factor in insulin release, inhibition of calcium flux could be responsible for a decreased sensitivity of the β cells to insulin releasing stimuli.

In this communication data will be presented which show that in pancreas pieces: (1) Both local anesthetics which bear a structural resemblance to acetylcholine (free tertiary amino group) and those that do not, inhibit glucose-stimulated insulin secretion. The inhibition resulting from local anesthetics which do not bear a structural resemblance to acetylcholine is partially overcome by the addition of excess calcium to the incubation media; (2) Local anesthetics with free tertiary amino groups inhibit Mecholyl-stimulated insulin whereas those without this structural feature do not. The inhibition is not overcome by excess calcium.

From the Departments of Pharmacology and Medicine, University of Arizona, College of Medicine, Tucson, Arizona.

METHODS AND MATERIALS

Animals: Adult male Sprague-Dawley rats were obtained from Camm Research, Norfolk, Virginia. Animals were fed ad libitum with diet consisting of water and Purina Lab Chow up to the time of their sacrifice. Animals weighing approximately 300 gm. were used for experiments involving whole pancreatic pieces.

Buffer: Buffers were made up fresh daily and contained, unless otherwise specified, sodium chloride, 100 mM; potassium chloride, 2 mM; calcium chloride, 2 mM; KH_2PO_4, 1.25 mM; magnesium sulfate, 1.25 mM; sodium bicarbonate, 26.2 mM; sodium pyruvate, 5 mM; sodium fumarate, 5.5 mM; sodium l-glutamate, 5 mM; and dextrose, 200 mg. per 100 ml. After preparation, buffers were gassed ten minutes with a 95:5 mixture of oxygen and carbon dioxide. In experiments requiring the measurements of insulin secretion, albumin was added to a final concentration of 0.2 per cent. All solutions were kept stoppered and on ice prior to use.

Pieces experiments: For experiments with pancreatic pieces, rats were stunned by a blow on the head followed by cervical dislocation. The splenic portion of the pancreas was immediately removed and transferred to a glass Petri dish of buffer over ice. All visible fat and lymph nodes were removed and the pancreas cut into approximately 100 pieces, each weighing about 5 mg. It was found that the use of smaller pieces enhances the insulin output per mg. of tissue. 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 ninety minutes at 100 cycles per minute. Antibody was added to the flasks to prevent the breakdown of insulin according to the method of Malaisse and coworkers.[19]

Insulin assay: Insulin was measured using a single antibody, cellulose column assay developed in our laboratory by Brendel et al.,* as a modification of the method of Malaisse.[19] Standard curves were run with each day's determinations.

Expression of results: All data were compared using the Student's *t* test for the comparison of two samples and 0.05 was chosen as the accepted level of significance. Data are presented as the mean ± standard error.

Sources: Holocaine and mepivicaine were gifts of Winthrop Laboratories. All other chemicals and pharmacologic agents were obtained from commercial sources. Guinea pig anti-porcine insulin anti-serum was provided by Dr. Peter Wright of the Pharmacology Department, University of Indiana. Porcine insulin for standards in the insulin assay was a gift of the Eli Lilly Company.

*Brendel, K., Stocks, B., Bressler, R. (In preparation).

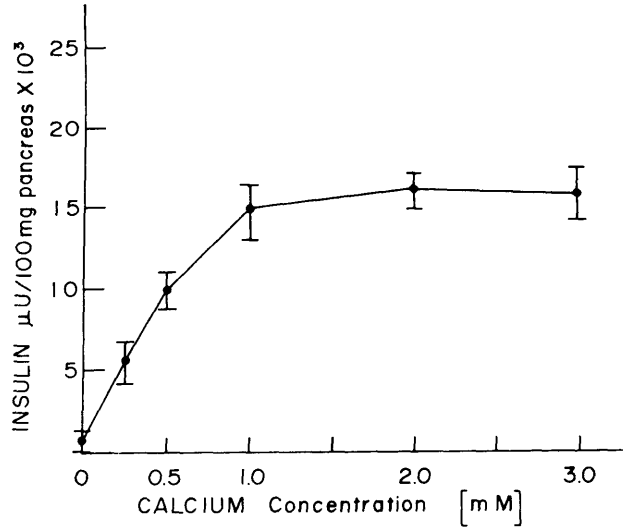


FIG. 1. The effect of calcium concentration on insulin secretion by pancreas pieces. Each point represents five determinations (six to ten pancreas pieces per determination).

RESULTS

Calcium concentration and in vitro insulin secretion

It has been shown that the stimulation of insulin secretion from either pancreatic pieces or the isolated perfused pancreas, by glucose or tolbutamide, is inhibited by the omission of calcium from the media.[12,13,20] The effect of calcium concentration on glucose-induced insulin secretion by pancreas pieces is shown in figure 1. Insulin output was progressively increased as the calcium concentration of the incubating media was raised from 0 to 1.0 mM. The level of insulin secretion then plateaued at calcium concentrations between 1 and 3 mM. Calcium concentrations above 6 mM resulted in inhibition of glucose-induced insulin secretion (data not shown).

Effects of cholinergic agonists and antagonists on insulin secretion

The data presented in table 1 show the effect of cholinergic agonists and antagonists on insulin secretion. β-methylcholine (Mecholyl) is a pharmacologic agent whose activity is confined to stimulation of parasympathetic muscarinic receptors. Mecholyl augmented insulin secretion by the pancreas pieces. Physostigmine is a cholinesterase inhibitor which exerts its effects by

TABLE 1

Cholinergic-stimulated insulin secretion*

Experiment	Insulin μU./100 mg. pancreas	P
Control	14,200 ± 1,100	—
Mecholyl 2×10^{-3} μM/ml.	21,400 ± 1,650	< 0.01
Physostigmine 8×10^{-3} μM/ml.	17,700 ± 740	< 0.05
Atropine 2×10^{-3} μM/ml.	13,500 ± 1,540	> 0.5
Mecholyl 2×10^{-3} μM/ml. + Physostigmine 8×10^{-3} μM/ml.	28,400 ± 1,300	< 0.01
Mecholyl 2×10^{-3} μM/ml. + Atropine 2×10^{-3} μM/ml.	11,800 ± 1,250	> 0.5

* Each experimental group consisted of six separate determinations. Calcium concentration 1.0 μM/ml.

virtue of its capacity to protect endogenously generated acetylcholine. The stimulatory effect of physostigmine on insulin secretion suggests intact muscarinic (neuroeffector) innervation of the pancreas pieces. Mecholyl plus physostigmine resulted in a stimulation of insulin secretion which was greater than that exerted by either agent alone. Atropine is a parasympathetic inhibitor which acts at muscarinic receptors to prevent access of acetylcholine. Atropine did not inhibit glucose-induced insulin secretion, but did inhibit Mecholyl-stimulated insulin secretion (table 1).

Effect of (±)—propranolol on insulin secretion

Studies on the role of extracellular calcium in catecholamine release from the perfused adrenal gland showed that propranolol, considered to be a classic β adrenergic receptor blocking agent, was capable of inhibiting the stimulatory effect of acetylcholine on catecholamine secretion.[14] It was concluded that the pharmacologic doses of propranolol used in these studies were acting as local anesthetics which inhibited intracellular calcium fluxes thereby depressing catecholamine release.[14,15]

Large doses of propranolol have also been found to inhibit the insulinotropic effects of glucose, tolbutamide, glucagon and cyclic 3′,5′ AMP in intact mice.[17,18] The (+) and (−) forms of propranolol were equipotent suggesting that the inhibiting effect was not due to the β adrenergic receptor blocking activity of propranolol because: (1) Whereas (−)—propranolol is an inhibitor of adenyl cyclase, (+)—propranolol is not.[21] However, both isomers are local anesthetics;[22-25] (2) (−) and (+)—propranolol both blocked the in vivo insulinotropic effect of cyclic AMP, the product of a β adrenergic stimulation. Therefore, these compounds had to be working at least in part at some site beyond the generation of cyclic AMP.

The effect of (±)—propranolol on insulin secretion by pancreas pieces is shown in table 2. Lower concentrations of propranolol (0.01 μM/ml.) had no effect on insulin secretion. High doses (1.0 μM/ml.) had a significant depressant effect on insulin secretion at low (0.5 mM) but not at higher (3.0 mM) calcium concentrations. These data are consistent with an inhibitory effect of propranolol on calcium fluxes in the pancreas resulting in an inhibition of glucose-stimulated insulin secretion.

TABLE 2

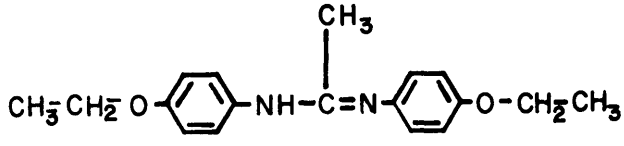
Effect of propranolol on insulin secretion*

Propranolol μM/ml.	Ca^{++} μM/ml.	Insulin μU./100 mg. pancreas	P
None	0.5	15,500 ± 1,300	—
0.01	0.5	16,000 ± 1,800	> 0.5
1.00	0.5	8,800 ± 580	< 0.01
1.00	3.0	14,200 ± 1,170	> 0.5

* Each experimental group consisted of six separate determinations.

Effects of holocaine on insulin secretion

Rubin and his coworkers have found that local anesthetics with a structural resemblance to acetylcholine inhibit medullary catecholamine secretion evoked by either acetylcholine or calcium, whereas local anesthetics which lack the free tertiary amino group inhibit solely by a depression of calcium flux.[14,15]



HOLOCAINE

Holocaine is a local anesthetic which lacks a free tertiary amino group (nonantiacetylcholine type). The data of table 3 show the effects of increasing concentrations of holocaine on glucose-induced insulin secre-

TABLE 3

Effect of holocaine on insulin secretion*

Holocaine μM/ml.	Ca^{++} μM/ml.	Insulin μU./100 mg. pancreas	P
None	0.50	10,700 ± 570	—
10^{-3}	0.50	9,800 ± 1,080	> 0.5
5×10^{-2}	0.50	6,100 ± 450	< 0.01
1	0.50	3,800 ± 980	< 0.01
None	3.0	19,600 ± 1,300	—
10^{-3}	3.0	18,700 ± 790	> 0.5
5×10^{-2}	3.0	16,800 ± 1,880	> 0.5
1	3.0	8,900 ± 660	< 0.01

* Each experimental group consisted of six separate determinations.

TABLE 4

Anticholinergic activity of holocaine and propranolol*

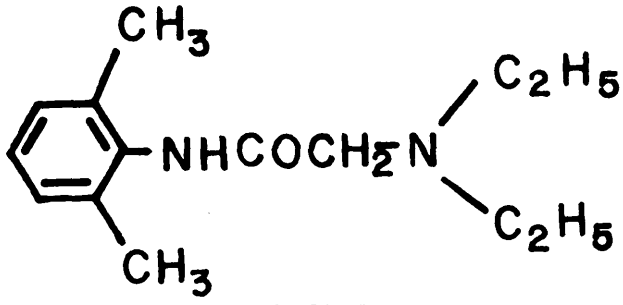
Experiment	Insulin μU./100 mg. pancreas	P Control	P Mecholyl
Control	12,500 ± 1,450	—	< 0.01
Mecholyl 2×10^{-3} μM/ml.	27,300 ± 1,600	< 0.01	—
Holocaine 1 μM/ml.	8,800 ± 1,080	< 0.01	—
Propranolol 1 μM/ml.	9,600 ± 590	< 0.01	—
Mecholyl 2×10^{-3} μM/ml. + Propranolol 1 μM/ml.	29,600 ± 1,150	< 0.01	> 0.5
Mecholyl 2×10^{-3} μM/ml. + Holocaine 1 μM/ml.	30,100 ± 1,350	< 0.01	> 0.5

* Each experimental group consisted of six separate determinations. Calcium concentration 0.5 μM/ml.

tion by pieces of pancreas at two different calcium concentrations. Significant depression of insulin secretion was obtained at a holocaine concentration of 5 x 10^{-2} mM when the calcium concentration was 0.5 mM, but not when the calcium concentration was raised to 3.0 mM. These data are consistent with an inhibitory effect of holocaine on calcium flux resulting in a depression of glucose-induced insulin secretion.

Effect of holocaine and propranolol on Mecholyl-stimulated insulin secretion

The data of table 4 show that holocaine and propranolol have inhibitory effects on insulin secretion, whereas Mecholyl stimulates secretion. Moreover, neither holocaine or propranolol, used in concentrations which resulted in depression of baseline glucose-induced insulin secretion, inhibited the stimulatory effect of Mecholyl.



LIDOCAINE

Effect of lidocaine on insulin secretion

Lidocaine is a local anesthetic with a free tertiary amino group. The inhibitory effect of lidocaine on insulin secretion is shown in table 5. The inhibition of glucose-induced insulin secretion resulting from lidocaine was apparent at calcium concentrations of 0.25 and 1.0 mM. Quantitative differences in degree of inhibition by lidocaine at the two concentrations of calcium were not discernible from these data.

TABLE 5

Lidocaine and calcium concentration*

Lidocaine μM/ml.	Ca^{++} μM/ml.	Insulin μU./100 mg. pancreas	P
None	0.25	5,300 ± 330	< 0.01
10^{-3}	0.25	2,850 ± 700	
None	1.0	12,700 ± 1,200	< 0.01
10^{-3}	1.0	9,300 ± 830	

* Each experimental group consisted of six separate determinations.

Lidocaine concentration and insulin secretion

The effect of increasing concentrations of lidocaine on insulin secretion is shown in table 6. Insulin secretion was depressed at a lidocaine concentration of 10^{-3} mM, unaffected at 0.1 mM and stimulated at 0.5 mM.

When the stimulatory dose of lidocaine (0.5 mM) was incubated in the presence of 2 x 10^{-3} mM atropine, insulin secretion was reduced to the level obtained in the presence of 10^{-3} mM lidocaine. These data are consistent with a cholinergic agonist effect of lidocaine at a concentration of 0.5 mM.

When 0.1 mM lidocaine, a dose which neither stimulated or inhibited insulin secretion, was incubated in the presence of 2 x 10^{-3} mM Mecholyl, an inhibition of the insulin stimulatory effect of Mecholyl resulted. These data are consistent with an anticholinergic effect of lidocaine at a concentration of 0.1 mM.

TABLE 6

Lidocaine and insulin secretion*

Experiment	Insulin μU./100 mg. pancreas	P
Control	13,780 ± 970	—
Lidocaine 10^{-3} μM/ml.	9,400 ± 1,150	< 0.01
Lidocaine 10^{-1} μM/ml.	12,800 ± 940	> 0.5
Lidocaine 5×10^{-1} μM/ml.	19,750 ± 710	< 0.01
Lidocaine 5×10^{-1} μM/ml. + Atropine 2×10^{-3} μM/ml.	8,300 ± 470	< 0.01
Mecholyl 2×10^{-3} μM/ml.	20,050 ± 1,300	< 0.01
Mecholyl 2×10^{-3} μM/ml. + Lidocaine 10^{-1} μM/ml.	11,800 ± 690	> 0.5

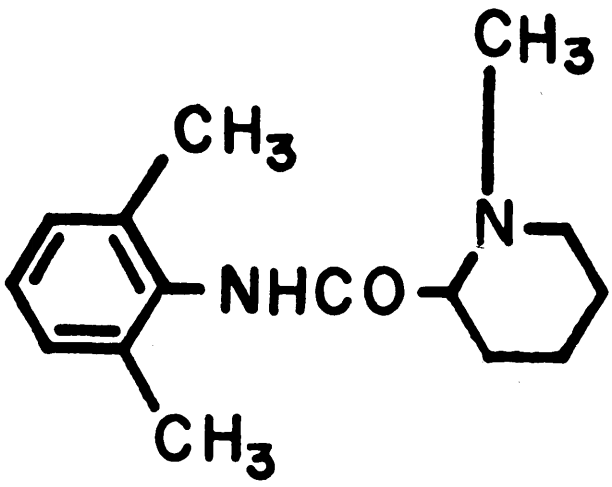
* Each experimental group consisted of six separate determinations. Calcium concentration 2.0 μM/ml.

TABLE 7

Mepivicaine and insulin secretion at various calcium concentrations*

Experiment	Insulin μU./100 mg. pancreas Ca++ Concentration μM/ml. 0.5	3.0	P
Control	8,800 ± 970	17,500 ± 1,150	—
Mepivicaine 10^{-3} μM/ml.	3,850 ± 510	12,300 ± 430	< 0.01
Mepivicaine 5×10^{-2} μM/ml.	9,100 ± 810	16,700 ± 1,300	> 0.5
Mepivicaine 1.5×10^{-1} μM/ml.	13,100 ± 1,300	28,300 ± 1,800	< 0.01
Mepivicaine 5×10^{-2} μM/ml. + Atropine 2×10^{-3} μM/ml.	4,300 ± 270	11,300 ± 820	< 0.01
Mepivicaine 1.5×10^{-1} μM/ml. + Atropine 2×10^{-3} μM/ml.	4,800 ± 1,110	10,090 ± 1,050	< 0.01

* Each experimental group consisted of six separate determinations.



MEPIVICAINE

Mepivicaine and insulin secretion

The effect of increasing concentrations of mepivicaine, a local anesthetic with a free tertiary amino group, on insulin secretion, at several different concentrations of calcium, is shown in table 7. Insulin secretion was inhibited at the lowest concentration of mepivicaine (10^{-3} mM), but unaffected at the intermediate mepivicaine concentration (0.15 mM). The inhibition of insulin secretion by the 10^{-3} mM mepivicaine was evident at both calcium concentrations (0.5 and 3.0 mM), but quantitative differences in the degree of inhibition by mepivicaine at the two calcium concentrations tested were not discernible from the data.

The addition of atropine to the incubation media with concentrations of mepivicaine which did not affect insulin secretion (5×10^{-2} mM) or which augmented it (0.15 mM) resulted in a depression of insulin secretion to levels obtained with the inhibitory concentration of mepivicaine (10^{-3} mM) alone.

Although insulin secretion was always greater at the higher calcium concentration, the qualitative nature of the results was not altered (table 7).

These data show that at low concentrations mepivicaine inhibits insulin secretion. At concentrations above 10^{-3} mM, mepivicaine acts as a cholinergic agonist as well as a local anesthetic. Atropine, an anticholinergic agent, unmasks the agonist effects of the higher concentrations of mepivicaine (5×10^{-2} mM, 0.15 mM) and explains the paradoxical disappearance of the inhibitory effect on insulin secretion and subsequent stimulation (table 7). Atropine removes the cholinergic effects of mepivicaine (lidocaine) and leaves the residual inhibitory effects, which are probably manifestations of the local anesthetic action of the drug.

Anticholinergic activity of mepivicaine

The data of table 8 show that at a concentration of 10^{-4} mM, mepivicaine does not inhibit insulin secretion, but does act as a potent anticholinergic agent. The anticholinergic activity of mepivicaine was evident at calcium concentrations of 0.5 and 3.0 mM. These results suggest that a low concentration of a local anesthetic of the antiacetylcholine type (free tertiary amino group) can inhibit the insulin response to cholinergic stimuli without markedly interfering with calcium flux.

DISCUSSION

The data presented show that different classes of local anesthetic influence insulin secretion by pancreatic pieces in different ways.

The nonantiacetylcholine type of local anesthetics (holocaine, propranolol) depress insulin secretion progressively as their concentrations in the incubating media are raised. This inhibition of secretion is more marked at lower calcium concentrations.

TABLE 8

Anticholinergic activity of mepivicaine*

Experiment	Insulin μU./100 mg. pancreas		P
	Calcium concentration μM/ml.		
	0.5	3.0	
Control	9,900 ± 820	16,500 ± 1,230	—
Mepivicaine 10^{-4} μM/ml.	10,070 ± 610	17,800 ± 1,370	< 0.5
Mecholyl 2×10^{-3} μM/ml.	22,700 ± 1,690	29,500 ± 1,710	< 0.01
Mecholyl 2×10^{-3} μM/ml. + Mepivicaine 10^{-4} μM/ml.	8,300 ± 760	15,900 ± 970	> 0.5

* Each experimental group consisted of six separate determinations.

The antiacetylcholine type of local anesthetics (lidocaine, mepivicaine) with charged tertiary amino groups have complex effects on insulin secretion. At low concentrations these drugs behave as anticholinergic agents manifested by their inhibition of Mecholyl-stimulated insulin secretion. They do not, however, depress basal glucose-induced insulin secretion. As the concentration of local anesthetic is raised an inhibition of glucose-stimulated insulin secretion occurs, which is not reversed by higher calcium concentrations. These data suggest that low concentrations of an antiacetylcholine type of local anesthetic can inhibit Mecholyl-stimulated insulin secretion without interfering with calcium movements. Complete blockade of Mecholyl-stimulated insulin secretion was found at calcium concentrations of both 0.5 and 3.0 mM (table 8). At still higher concentrations (0.05 to 1.0 mM) of local anesthetic the inhibitory effect on insulin secretion disappeared and was converted to a stimulatory effect, which was obviated by the addition of atropine to the incubation.

Higher concentrations of calcium lessened the inhibition of glucose-stimulated insulin secretion due to holocaine and propranolol (no charged nitrogens), whereas they did not significantly reverse the decrease due to lidocaine and mepivicaine (charged nitrogen atoms) or the decrease in Mecholyl-stimulated insulin secretion due to mepivicaine. Lidocaine and mepivicaine behaved as cholinergic agonists at higher concentrations. The transition of lidocaine and mepivicaine as first anticholinergic agents and later cholinergic agonists, as their concentrations are raised from low to high, is in contrast to atropine. Atropine has been postulated to produce its anticholinergic effects by attaching to the postganglion receptors and thus preventing the access of acetylcholine to these receptors.[26] Atropine blockage of cholinergic activation may be accompanied by a transient phase of stimulation of the receptor which occurs during the course of attachment. This brief agonist effect is obtained at lower levels of atropine. Higher concentrations of the antimuscarinic agent result in an inhibitory effect only.

This is in marked contrast to the effects obtained with the antiacetylcholine type of local anesthetic such as lidocaine and mepivicaine, which are antimuscarinic at lower concentrations and cholinergic agonists at higher concentrations.

The mechanism of the agonist or anticholinergic effects is not known, but appears to be independent of the calcium concentration. The two effects are manifested at different drug concentrations (10^{-2} versus 10^{-4} mM range) and may involve separate and distinct mechanisms. The anticholinergic effect may be the result of the action of the local anesthetics at the muscarinic receptor. The higher concentrations of local anesthetic which are agonistic show a graded stimulatory effect on insulin secretion. The possibility exists that these agonists are not acting as cholinergic stimulants at the muscarinic receptor, but rather as allosteric effectors at a site remote from the receptor. This type of attachment could lead to changes in receptor conformation altering the Km of the site for calcium and for acetylcholine.

The need for larger amounts of local anesthetic for the agonist effect has a precedent in the case of citrate (isocitrate) and its stimulation of long-chain fatty acid synthesis. Citrate at high concentrations causes an aggregation of the four subunits of purified acetyl CoA carboxylase and effects a many-fold stimulation of its activity. The enzyme is the rate limiting step in the over-all sequence, and fatty acid synthesis rates increase.[27]

The data do not permit us to say that the agonist effect is not at the muscarinic receptor, however, the antiacetylcholine type local anesthetics do have intact anticholinergic activity at concentrations where they are also agonists. Moreover, atropine presumably acting at

the muscarinic receptor does abolish the agonist effect of the local anesthetic.

Recent studies have shown that the secretion of insulin involves the packaging of the hormone into granules. These granules under the stimulus of glucose or other insulin secretagogues migrate to the cell membrane, where the granule and plasma membranes fuse resulting in a release of insulin.[28] It has been proposed that the migration of granules to the cell surface occurs on a structural framework of microtubules. This is supported by the observation that colchicine, which binds to microtubular protein, inhibits insulin secretion.[29] Both calcium and cyclic 3'5' AMP have been found to be involved in insulin secretion.[30] Although β blockade only diminishes the insulin response to β adrenergic stimulation, the lack of calcium inhibits insulin secretion in response to all insulin secretagogues.[31]

The role that calcium plays in insulin secretion is still obscure, but it has been postulated that it involves a calcium uptake process which requires sodium.[29] This is consistent with the stimulation of insulin secretion by ouabain, a compound which decreases the sodium-potassium ATPase,[32] resulting in an increase in intracellular sodium and with the inhibition of insulin secretion by diphenylhydantoin,[33,34] a compound which stimulates the ATPase resulting in a decrease in intracellular sodium.[35]

ACKNOWLEDGMENT

This work was supported in part by grants from the U.S. Public Health Service (AM14977 and HE13636). Dr. Brendel is an established investigator of the American Heart Association. Superb technical assistance was provided by Mrs. Linda Rice and Mrs. Brenda Stocks.

REFERENCES

[1] Esterhuizen, A. C., Spriggs, T. L. B., and Lever, J. D.: Nature of islet-cell innervation in the cat pancreas. Diabetes *17*:33, 1967.

[2] Coupland, R. E.: The innervation of the pancreas of the rat, cat, and rabbit as revealed by cholinesterase technic. J. Anat. *92*:143, 1958.

[3] Clark, A. G.: The influence of the vagus on the secretion of insulin. J. Physiol. *73*:297, 1931.

[4] Porte, D., Jr.: Beta adrenergic stimulation of insulin release in man. Diabetes *16*:150, 1967.

[5] Porte, D., Jr.: Sympathetic regulation of insulin secretion. Arch. Intern. Med. *123*:252, 1969.

[6] Turtle, J. R., and Kipnis, D. M.: An adrenergic receptor mechanism for the control of cyclic 3',5' adenosine monophosphate synthesis in tissues. Biochem. Biophys. Res. Commun. *28*:797, 1967.

[7] Porte, D., Jr., and Williams, R. M.: Inhibition of insulin release by norepinephrine in man. Science *152*:1248, 1966.

[8] Kaneto, A., Kosaka, K., and Nakao, K.: Effects of stimulation of the vagus nerve on insulin secretion. Endocrinology *80*:530, 1967.

[9] Frohman, L. A., Ezdinli, E. Z., and Javid, R.: Effect of vagotomy and vagal stimulation on insulin secretion. Diabetes *16*:443, 1967.

[10] Frohman, L. A.: The endocrine function of the pancreas. Ann. Rev. Physiol. *31*:353, 1969.

[11] Malaisse, W. F., Malaisse-Lagae, F., Wright, P. H., and Ashmore, J.: Effects of adrenergic and cholinergic agents on insulin secretion in vitro. Endocrinology *80*:975, 1967.

[12] Hales, C. W., and Milner, R. D. G.: The role of sodium and potassium in insulin secretion from rabbit pancreas. J. Physiol. (London) *194*:725, 1968.

[13] Grodsky, G. N., and Bennett, L. L.: Cation requirements for insulin secretion in the isolated perfused pancreas. Diabetes *15*:910, 1966.

[14] Jaanus, S. D., Miele, E., and Rubin, R. P.: The analysis of the inhibitory effect of local anesthetics and propranolol on adrenomedullary secretion evoked by calcium or acetylcholine. Brit. J. Pharmacol. Chemother. *31*:319, 1967.

[15] Miele, E., and Rubin, R. P.: Further evidence for dual action of local anesthetics on the adrenal medulla. J. Pharmacol. Exp. Ther. *161*:296, 1968.

[16] Bressler, R., Vargas-Cordon, M., and Lebovitz, H. L.: Tranylcypromine, a potent insulin secretagogue and hypoglycemic agent. Diabetes *17*:617, 1968.

[17] Bressler, R., Vargas-Cordon, M., and Brendel, K.: Studies on the role of adenylcyclase in insulin secretion. Arch. Intern. Med. *123*:248, 1969.

[18] Bressler, R., Vargas-Cordon, M., and Brendel, K.: The inhibition of insulin secretion by aryl-substituted secondary aminoethanols. Diabetes *18*:262, 1969.

[19] Malaisse, W. J., Malaisse-Lagae, F., and Wright, P. H.: A new method for the measurement in vitro of pancreatic insulin secretion. Endocrinology *80*:99, 1967.

[20] Milner, R. D. G., and Hales, C. N.: Cations and secretion of insulin. Biochim. Biophys. Acta *150*:165, 1967.

[21] Howe, R.: Structure activity relationships of some β adrenergic blocking agents. Biochem. Pharmacol. *12* (Suppl. 1):85, 1963.

[22] Levy, J. W., and Richards, V.: Inotropic and chronotropic effects of a series of β adrenergic blocking drugs. Some structure-activity relationships. Proc. Soc. Exp. Biol. Med. *122*:373, 1966.

[23] Ablad, B., Brogard, M., and Ek, L.: Pharmacological properties of H56/28 a β adrenergic receptor antagonist. Acta Pharmacol. (Kobenhavn) *25* (Suppl. 2):9, 1967.

[24] Lucchesi, B. R.: The effects of Pronethalol and its dextro isomer on experimental cardiac arrhythmias. J. Pharmacol. Exp. Ther. *148*:94, 1965.

[25] Morales-Aquilera, A., and Vaughn-Williams, E. M.: The effects on cardiac muscle of β receptor antagonists in relation to their activity as local anesthetics. Brit. J. Pharmacol. *24*:332, 1965.

[26] Cutting, W.: Handbook of Pharmacology, Third Edition. New York, Appleton-Century Crofts, 1967, p. 441.

[27] Waite, M., and Wakil, S. J.: Studies on the mechanism of fatty acid synthesis, XII acetyl coenzyme A carboxylase. J. Biol. Chem. *237*:2750, 1962.

[28] Lacy, P. E.: The pancreatic beta cell structure and function. New Eng. J. Med. *276*:187, 1967.

[29] Lacy, P. E., Howell, S. L., Young, D. A., and Fink, C. J.: New hypothesis of insulin secretion. Nature (London) *219*:1177, 1968.

[30] Rasmussen, H.: Cell communication, calcium ion and cyclic adenosine monophosphate. Science *170*:404, 1970.

[31] Kizer, J. S., and Bressler, R.: Drugs and the mechanism of insulin secretion. Advances Pharmacol. Chemother. *7*:91, 1969.

[32] Skow, J.: Enzymatic basis for active transport of Na^+ and K^+ across cell membranes. Physiol. Rev. *45*:596, 1965.

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

[34] Levin, S. R., Booker, J., Smith, D. F., and Grodsky, G. M.: Inhibition of insulin secretion by diphenylhydantoin in the isolated perfused pancreas. J. Clin. Endocr. *30*:400, 1970.

[35] Festoff, B. W., and Appel, S. H.: Effect of diphenylhydantoin on synaptosome sodium-potassium-ATPase. J. Clin. Invest. *47*:2752, 1968.

Effects of Dietary Changes on Kidney Metabolism

H. M. Tepperman, P. Fabry, and J. Tepperman have now produced data on the effect of various feeding schedules on kidney cortex enzymes, gluconeogenesis, water intake, and urinary excretion patterns (J. Nutrition *100*:837, 1970). Adult rats were first trained to eat on alternate days for two weeks, and then entered a program of fasting for two days, followed by one twenty-four hour period of ad libitum feeding. This second phase lasted for three to eleven weeks, but it is not made clear in the experimental data given whether length of time on this regimen affected the results. Two-thirds of the rats were able to maintain or slowly increase their body weights on this schedule and any who did not were rejected. In the studies on water balance, rats were either fed for two hours daily or on alternate days. The response of both liver and kidney enzymes to a high fat or high protein diet was also studied. The perfused kidney preparation was used to measure gluconeogenesis with amino acids, lactate, pyruvate, and glycerol provided simultaneously as substrates.

No dietary manipulation caused any change in the activities of the dehydrogenases of the hexosemonophosphate shunt or in the NADP malic enzyme. This is in contrast with previous studies from the same laboratory on these enzymes in liver (Tepperman and Tepperman, Fed. Proc. *29*:1284, 1970). Control rats and rats who were trained to eat every third day were fasted for forty-eight hours and then refed. After the fast, pyruvate carboxylase activity in kidney cortex was elevated in the trained but not in the control rats, and these high levels persisted in the trained rats even after twenty-four hours of refeeding. The response of PEPCK was different. Activity of this enzyme increased after fasting in both the control and the trained rats, and the high levels also persisted in both groups after refeeding. There was no significant difference between trained and control rats in the magnitude of the response to fasting. After twenty-four hours of refeeding the PEPCK had not returned to the pre-fast level.

Although the high protein diet caused an increase in liver and kidney weight as well as an increase in liver protein, there was no increase in kidney cortex protein. This diet produced an increase in liver and kidney PEPCK, but not pyruvate carboxylase. The high fat diet led to increases in PEPCK in both liver and kidney.

The elevated levels of renal pyruvate carboxylase and PEPCK were associated with increased rates of renal gluconeogenesis. In an attempt to correct for the smaller size of the trained rats, controls of the same weight or of the same age were used, but renal gluconeogenesis in the trained rats was higher than in either of the two groups of controls, as were PEPCK and

(*Continued on page* 733)