Tris (Hydroxymethyl) Aminomethane (THAM) Induced Stimulation of Insulin Release by Islets of Langerhans Previously Isolated from Rat Pancreas

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SUMMARY

Islets isolated from rat pancreas were studied in regard to release of immunoreactive insulin (IRI) as influenced by changes in glucose concentration and pH and type of buffer in the incubation media. The pH of the media was varied from 7.4 to 9.0. For phosphate and bicarbonate buffered media, change in pH did not result in significant change in IRI release rates. For media containing the pH buffer and chelating compound THAM (Tris [hydroxymethyl] aminomethane), IRI release increased significantly with increase in either THAM concentration or pH. Since increase in pH results in increase in that fraction of THAM which is in the unionized, therefore presumably more readily penetrating form, it is postulated that THAM molecules penetrate and act at intracellular loci to induce insulin release, possibly by chelating Zn complexed with insulin in beta cell granules. DIABETES *19*:559-62, August, 1970.

Hypoglycemia may be induced in both humans[1,2] and experimental animals[2,3] by intravenous administration of the pH buffer tris (hydroxymethyl) aminomethane, also known as THAM, Tris, Tromethane and 2-amino-2-hydroxymethyl-1, 3- propanediol. Tarail and Bennett[2] found that THAM had no significant effect on blood glucose levels of pancreatectomized dogs and therefore postulated that when it induces hypoglycemia it does so by stimulating insulin release. Zaharko[3] provided evidence that in the dog THAM does indeed induce release of immunoreactive insulin (IRI): (a) following THAM intravenous administration the plasma IRI level was temporarily elevated, and (b) the induced increase in plasma IRI level was much greater when a given amount of THAM was administered via the pancreaticoduodenal artery than by a peripheral vein. Zaharko also found that a THAM solution of pH 10 was much more effective than an equimolar THAM solution of pH 7.4 in inducing IRI release. With increase in pH the ionization of THAM decreases, resulting presumably in increase in both THAM penetration and its stimulation of IRI release.

The possibility always exists that any given action of a compound administered systematically may be indirect. Experiments therefore have been performed to determine what effects THAM might have on IRI release by islets of Langerhans previously isolated from rat pancreas.

MATERIAL AND METHODS

Tris (hydroxymethyl) aminomethane (THAM; Tris) in both its basic and neutralized forms was obtained from Sigma Company, St. Louis, Mo. The insulin I-125 used in the insulin immunoassays was obtained from Abbott Company, North Chicago, Illinois. We are greatly indebted to Dr. Peter Wright of this same Department for guinea pig anti-insulin serum (AIS), and to Dr. Mary Root of The Eli Lilly Company, Indianapolis, Indiana, for the bovine insulin used as standard. Measurement of immunoreactive insulin (IRI) was made as described by Zaharko and Beck.[4] When necessary, samples were neutralized to pH 7.4, standards were prepared in incubation fluids of pH 7.4, and both test samples and standards were diluted at least ten times with the THAM[4] before the immunoassay procedure proper was done. Each sample was assayed in duplicate. Values accepted were those obtained using the straight line portion of an immunoassay curve. Wistar strain rats, both male and female, of 300 to 400

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TABLE 1

Immunoreactive insulin (IRI) release by islets of Langerhans, rat pancreas origin: Variations with changes in glucose concentration, in the pH buffer used, and in the pH of the incubation medium

Data column	Composition of incubation medium			n†	IRI release (mμg./hr./5 islets; Mean±S.E.M.)	Differences between means significant ($p < .01$) or not significant (NS; $p > .01$)‡
	Buffer used*	mg./100 ml. glucose	pH			
a	PO_4	0	7.4	11	2 ± 0.4	I. Islets in PO_4 versus islets in HCO_3; 30 mg./100 ml. glucose: PO_4 (b-f incl.) < HCO_3 (n-r incl.)
b	PO_4	30	7.4	4	6	
c	PO_4	30	7.8	3	5	
d	PO_4	30	8.2	3	4	
e	PO_4	30	8.6	3	4	II. Islets in 30 mg./100 ml. glucose versus islets in 300 mg./100 ml. glucose: A. PO_4: 30 (b-f incl.) < 300 (h-l incl.) B. HCO_3: 30 (n-r) incl.) < 300 (t-x incl.)
f	PO_4	30	9.0	3	4	
	b-f inclusive: 30			16	4.7± 0.8	
g	PO_4	100	7.4	4	16	
h	PO_4	300	7.4	15	66 ± 5.4	
i	PO_4	300	7.8	15	71 ±13.2	
j	PO_4	300	8.2	12	63 ±11.2	
k	PO_4	300	8.6	16	84 ± 6.5	III. Islets in buffer at pH 7.4 versus islets in buffer at pH 9.0; 300 mg./100 ml. glucose: A. PO_4: 7.4 (h) vs. 9.0 (l), NS B. HCO_3: 7.4 (t) vs. 9.0 (x), NS
l	PO_4	300	9.0	10	79 ±11.7	
	h-l inclusive: 300			68	73 ± 4.4	
m	HCO_3	0	7.4	4	16	
n	HCO_3	30	7.4	6	20 ± 3.3	
o	HCO_3	30	7.8	5	18 ± 2.4	
p	HCO_3	30	8.2	4	18	
q	HCO_3	30	8.6	3	16	
r	HCO_3	30	9.0	4	20	
	n-r inclusive: 30			22	20 ± 1.4	
s	HCO_3	100	7.4	4	40	
t	HCO_3	300	7.4	12	65 ± 8.7	
u	HCO_3	300	7.8	11	70 ±16.9	
v	HCO_3	300	8.2	8	42 ± 4.2	
w	HCO_3	300	8.6	11	47 ± 6.0	
x	HCO_3	300	9.0	12	57 ± 5.8	
	t-x inclusive: 300			54	57 ± 4.0	

*PO_4 = phosphate buffer of indicated pH; HCO_3 = bicarbonate buffer of indicated pH.
†n = number of separate incubations, each done using islets from a different rat pancreas.
‡Letters are taken from Data column and indicate means or groups of means compared.

gm. body weight, and obtained from Laboratory Supply Company, Indianapolis, provided pancreatic tissue. Islets of Langerhans were isolated from each excised pancreas as described by Lacy and Kostianowsky.[5] Five islets were used per incubation tube. Each fluid in which islets were suspended was prepared to contain total ions at approximately 300 millimolar concentration, with glucose amount and pH as indicated in the tables. Each phosphate buffer solution contained altogether 13 mmoles of a mixture of mono- and dibasic phosphate adjusted to give the desired pH, 133 mmoles NaCl, 4 mmoles KCl, 0.9 mmoles $CaCl_2$, 0.5 mmoles $MgCl_2$. Krebs bicarbonate solutions were prepared to keep the millimolar sum of NaCl and $NaHCo_3$ constant, and to have the pH adjusted to the desired level by aeration of the solution with 95 per cent O_2, 5 per cent CO_2. Each 0.05 M THAM solution of stated pH was obtained by mixing one volume 0.15 M THAM of that pH with two volumes phosphate buffer of the same pH; each 0.10 M THAM solution was prepared similarly using two volumes 0.15 M THAM desired pH and one volume phosphate buffer, same pH. The THAM or THAM-phosphate solutions were aerated at 37° C. with 100 per cent O_2.

TABLE 2

Effects of THAM on IRI release by islets of Langerhans, rat pancreas origin

Data column	Composition of incubation medium: THAM molar conc.*	mg./100 ml. glucose	pH	n†	IRI release (mμg./hr./5 islets; Mean±S.E.M.)	Differences between means significant ($p < .01$) or not significant (NS, $p > .01$)‡
a	Zero	30	7.4	4	6	I. THAM concentration varied; 300 mg./100 ml. glucose; pH held constant: A. pH 7.4: Zero (m) & .05(n) < .10(o) & .15(p) B. pH 8.2: Zero (q) < .05 (r) & .15 (t) < .10 (s) C. pH 9.0: Zero (u) < .05 (v) < .15 (x) < .10 (w)
b	.05	30	7.4	4	4	
c	.10	30	7.4	4	4	
d	.15	30	7.4	7	12± 1.6	
e	Zero	30	8.2	3	4	
f	.05	30	8.2	4	16	
g	.10	30	8.2	4	38	
h	.15	30	8.2	7	40± 5.0	
i	Zero	30	9.0	3	4	II. pH varied, THAM concentration held constant: A. 30 mg./100 ml. glucose, .15 M THAM: 7.4 (d) < 8.2 (h) < 9.0 (l) B. 300 mg./100 ml. glucose: 1. .05 M THAM: 7.4 (n) < 8.2 (r) & 9.0 (v) 2. .10 M THAM: 7.4 (o) < 8. 2 (s) < 9.0 (w) 3. .15 M THAM: 7.4 (p) < 8.2 (t) < 9.0 (x)
j	.05	30	9.0	4	20	
k	.10	30	9.0	4	140	
l	.15	30	9.0	7	248±14.7	
m	Zero	300	7.4	15	66± 5.4	
n	.05	300	7.4	7	60± 2.6	
o	.10	300	7.4	5	86± 5.4	
p	.15	300	7.4	12	97± 4.0	
q	Zero	300	8.2	12	63±11.6	III. Mg./100 ml. glucose varied; THAM at .15 M; pH held constant: A. pH 7.4: 30 mg./100 ml. (d) < 300 mg./100 ml. (p) B. pH 8.2: 30 mg./100 ml. (h) < 300 mg./100 ml. (t) C. pH 9.0: 30 mg./100 ml. (l) vs. 300 mg./100 ml. (x), NS
r	.05	300	8.2	8	147± 8.5	
s	10	300	8.2	9	189± 8.0	
t	.15	300	8.2	12	134± 3.5	
u	Zero	300	9.0	10	79±11.7	
v	.05	300	9.0	7	150±13.2	
w	.10	300	9.0	11	292± 7.3	
x	.15	300	9.0	14	220± 6.1	

*Zero THAM molar concentration indicates that phosphate buffer was used; data are the same as those presented in table 1; .05 M THAM indicates that one part of THAM buffer of that pH was mixed with two parts phosphate buffer, same pH; .10 M THAM that two parts THAM buffer of that pH were mixed with one part phosphate buffer, same pH.
†n = number of separate incubations, each done using islets from a different rat pancreas.
‡Letters are taken from Data column and indicate means compared.

RESULTS

Effects of glucose concentration and of pH on IRI release, with pH governed by use of a phosphate or bicarbonate buffer:

The findings are given in table 1. IRI release increased with increase in glucose concentration in fashion similar to that reported by others.[5,6] (cf. II under Differences between means [Dbm] column). At 30 mg./100 ml. glucose concentration, islets exhibited somewhat greater IRI release when incubated in Krebs bicarbonate than when incubated in phosphate buffer (cf. I under Dbm column). Change in pH of either the phosphate or the bicarbonate incubation medium, over the range 7.4 to 9.0 (glucose concentration 300 mg./100 ml.) did not result in significant change in IRI release rate (cf. III under Dbm column).

Effects of THAM on IRI release by incubated islets:

The findings are given in table 2. With glucose concentration held at 300 mg./100 ml. and pH at 7.4 or 8.2 or 9.0, IRI release was usually significantly greater when THAM was present than when it was absent, and usually significantly greater when THAM concentration was 0.10 or 0.15 M than when it was 0.05 M (cf. I under Dbm column). However, at both pH 8.2 and pH 9.0, THAM was apparently more effective in stimulating IRI release when used at 0.10 M than when used at 0.15 M concentration. At any given THAM concentration (0.05, 0.10 or 0.15 M), IRI release increased significantly with increase in pH from 7.4 to 8.2 and/or 9.0 (cf. II under Dbm column). As in the table 1 experiments, changes from 30 to 300 mg./100 ml. glucose resulted in significant increase in IRI release, except when as with 0.15 M THAM at pH 9.0, IRI release in the presence of 30 mg./100 ml. glucose was already very high (cf. III under Dbm column).

DISCUSSION

THAM has been used in the management of clinical acidosis, particularly in the newborn,[7-10] burn patients[11] and those acutely hypercapneic.[12] Severe hypoglycemia has been reported occasionally in a THAM-treated patient.[2] The findings of the present study substantiate the hypothesis advanced earlier by Tarail and Bennett[2] and concurred in by Zaharko,[3] that THAM-induced hypoglycemia is due in the main to the effect of THAM to induce endogenous insulin release. It is probable that THAM-induced in vivo insulin release will be temporary in nature, however, and hypoglycemia may be prevented by careful monitoring of the blood glucose level and through appropriate glucose administration.

It is stated that at 37° C. THAM has a pK of about 7.81, and hence may be expected to be approximately 38 per cent unionized at pH 7.4 and about 94 per cent unionized at pH 9.0. If only the unionized form were to penetrate the cells, the intracellular concentration at pH 9.0 would be about twice that at pH 7.4. It seems likely, therefore, that the greater effectiveness of this compound to induce insulin release when used at pH 9.0 was due in the main to a greater penetration of THAM molecules into beta cells, and that this compound induces insulin release by some actions(s) occurring at intracellular loci.

One possibility is that THAM molecules chelate zinc, and thus split up insoluble insulin-zinc complexes present within beta cell granules, making this insulin available for release. Maske[13] has presented evidence that zinc forms aggregates with insulin within the granules, and has postulated that at least some insulin-releasing agents act by chelating zinc to a greater extent than does insulin. Okamoto and Kiwanishi[14] have provided histochemical evidence that zinc is present within beta cell secretory granules and their surrounding sacs. Hall et al.[15] have reported that amino acid-metal complexes form more readily at alkaline than at neutral pH, as might be expected from the law of mass action. Chambers[16] found that visible cytoplasmic inclusions, previously vitally stained with methyl red or neutral red, were much more susceptible to pH altering effects of penetrating acids and bases respectively than was the cytoplasm proper. Therefore, it is possible that THAM acts to induce insulin release, not only by competing with insulin for metallic ions which help to hold the insulin in an insoluble form inside granules, but also by rendering these granules more alkaline, so that they are more susceptible to having their contained metallic ions chelated by the THAM.

ACKNOWLEDGMENT

We are grateful to Dr. Daniel S. Zaharko for advice. This investigation was supported by U.S. Public Health Service Grant AM 05980 and a grant from Eli Lilly Co.

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