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The influence of chlorpropamide pre-treatment on insulin and growth hormone release after arginine

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THE hypoglycaemic effect of the sulphonylurea family of drugs was long suspected to be secondary to endogenous insulin release from the pancreatic islets. This was confirmed by Yalow *et al.* (1960) who showed a rise in peripheral plasma concentration of insulin after tolbutamide administration to normal subjects. More recently, Coore & Randle (1964) showed that, when isolated segments of rabbit pancreas were incubated in the presence of tolbutamide, there was additional release of insulin into the medium. Further evidence that the sulphonylurea drugs increase pancreatic islet-cell activity emerged from the thorough studies of Fajans *et al.* (1963) and Floyd *et al.* (1963). These authors showed that, whereas the amino acid, L-leucine, induced only a modest rise in peripheral plasma insulin concentration in normal subjects, the response was dramatically enhanced by pre-treatment of these subjects with chlorpropamide.

Floyd *et al.* (1966) have since shown that infusion of a number of amino acids, including L-arginine, is followed by a rise in peripheral plasma insulin levels. We have confirmed that arginine infusion initiates a rise in plasma insulin concentration. There is, in addition, a rise in plasma HGH concentration after arginine, a response which is quantitatively greater in the female than in the male (Merimee, Lillicrap & Rabinowitz, 1965; Merimee, Burgess & Rabinowitz, 1966). In the light of the considerable influence of chlorpropamide on leucine-initiated insulin release, we were led to explore the effect of this sulphonylurea on arginine-initiated hormonal release. We chose a dosage schedule of arginine monochloride which usually produces only a modest increment in plasma insulin in control subjects. Plasma glucose, insulin and HGH responses following a standard load of L-arginine to seven normal males before and after chlorpropamide pre-treatment form the basis of this report.

Methods

All studies were performed during the morning hours after an overnight fast. A venous catheter or an indwelling needle was placed percutaneously into a forearm vein. After two sets of control samples had been drawn, arginine monochloride (30 g) was infused over 30 min. Intermittent samples were taken over the next 2 hr. All subjects were then placed on chlorpropamide 0·5 g daily for 4–6 days, after which they were again challenged with arginine monochloride. Glucose was measured by a glucose oxidase method; plasma insulin and HGH were measured by radioimmunoassay using the methods of Yalow & Berson (1960) and Roth *et al.* (1963) with only minor modifications.

Results

Results are shown in Tables 1–4 and in Figs. 1–4.

TABLE 1

Plasma glucose concentration after arginine infusion*

Subject	Control								After chlorpropamide pre-treatment							
Time (min):	−15	0	15	30	45	60	90	120	−15	0	15	30	45	60	90	120
L.A.	88	97	—	67	—	77	82	99	55	59	78	80	69	72	69	68
J.L.	95	—	120	117	95	87	87	101	81	81	101	112	102	87	—	90
R.L.	90	93	116	117	84	77	90	96	81	81	111	110	83	73	97	89
C.H.	94	—	112	123	114	101	—	100	86	93	106	101	95	92	83	86
C.Z.	97	111	105	118	71	69	78	105	90	99	105	132	82	77	110	102
J.T.	102	86	120	119	97	65	78	77	73	84	93	103	90	68	77	79
R.B.	99	84	112	119	110	87	85	106	63	63	68	79	71	75	73	91

* Arginine monochloride infused from time zero to time 30. All values are mg/100 ml.

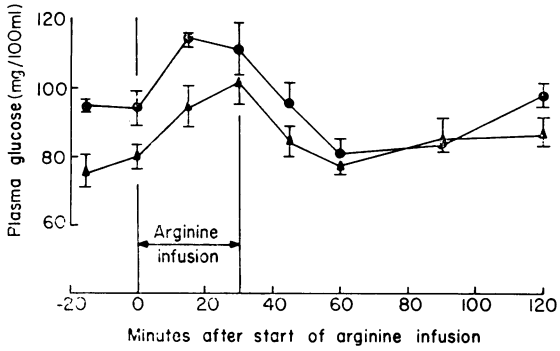

FIG. 1. Plasma glucose concentrations in seven male subjects before, during and after arginine infusion, without pre-treatment (●) and after chlorpropamide pre-treatment (▲). All values are means ± SE of mean.

Glucose (Table 1, Fig. 1)

The observed changes after arginine were qualitatively similar in the initial study and after the subjects had received chlorpropamide: an early hyperglycaemia followed by a fall to or below basal values (Fig. 1). After chlorpropamide pre-treatment there was a downward trend in basal glucose concentration (a fall in mean basal glucose from 94·6 to 77·8 mg/100 ml) and the peak glucose values achieved after arginine were not as high as in the initial study.

Insulin (Tables 2 and 3, Figs. 2 and 3)

Basal plasma insulin levels were modestly elevated after chlorpropamide pre-treatment. The plasma insulin response initiated by arginine infusion was not dissimilar before and after chlorpropamide therapy. We were unable to demonstrate any consistent effect of sulphonylurea pre-treatment on insulin responsiveness to arginine when the data were analysed in the following three ways: (a) when the mean insulin response was compared (Fig. 2); (b) when the mean change in peripheral insulin levels was compared (Table 3); and (c) when the individual plasma insulin responses to arginine were examined (Fig. 3).

From the last analysis, there was a suggestion

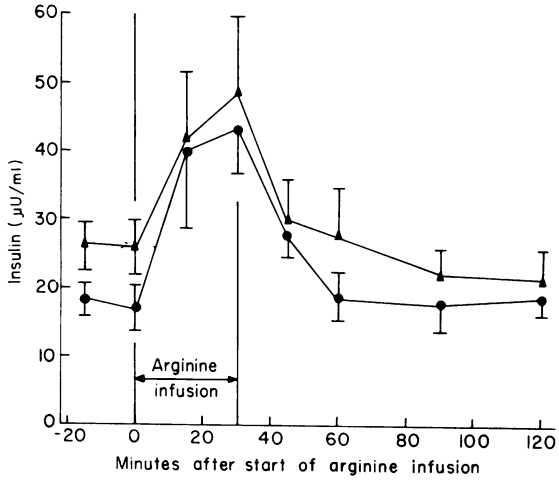

FIG. 2. Plasma insulin concentrations in seven male subjects before, during and after arginine infusion, without pre-treatment (●) and after chlorpropamide pre-treatment (▲). All values are means ± SE of mean.

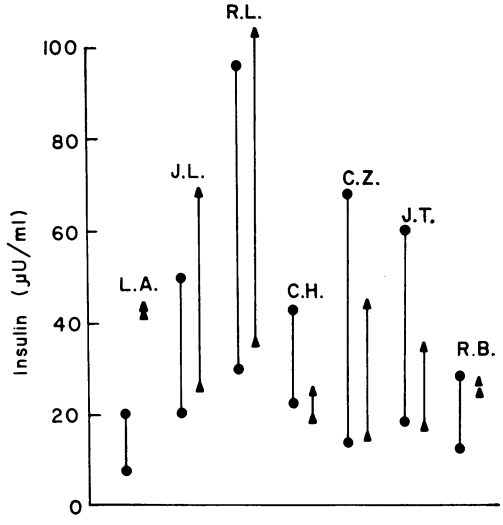

FIG. 3. A comparison of response of plasma insulin concentration to arginine in seven subjects (basal and peak insulin levels shown) without pre-treatment (●) and after chlorpropamide pre-treatment (▲).

TABLE 2

Plasma insulin concentration after arginine infusion*

Subject	Control								After chlorpropamide pre-treatment							
Time (min):	−15	0	15	30	45	60	90	120	−15	0	15	30	45	60	90	120
L.A.	8	8	—	20	—	14	10	12	40	42	42	43	25	19	26	22
J.L.	22	20	34	50	31	38	35	28	26	26	62	69	55	56	—	—
R.L.	28	30	96	33	34	20	22	24	36	36	90	103	50	54	36	40
C.H.	20	22	28	43	26	18	—	12	19	20	25	25	18	15	13	14
C.Z.	16	13	32	68	28	14	10	20	14	15	24	44	22	14	28	19
J.T.	20	18	31	60	35	16	18	14	18	16	28	34	20	18	12	12
R.B.	14	12	20	28	12	10	12	18	30	27	24	22	20	16	16	20

* Arginine monochloride infused from time zero to time 30. All values are μU/ml.

TABLE 3
Change in plasma insulin concentration following arginine infusion*

	Time (min)					
	15	30	45	60	90	120
Without pre-treatment						
Mean	+20·6	+25·2	+8·1	+0·7	+0·4	+0·4
SEM	9·4	6·6	2·6	3.2	3·0	2·4
Following chlorpropamide pre-treatment						
Mean	+16·2	+22·5	+3·9	+1·3	−4·4	−4·9
SEM	8·0	9·8	5·6	6·7	4·2	3·6

* Arginine monochloride infused from time zero to time 30. Data were analysed by comparing each individual's plasma insulin levels after arginine with his basal insulin concentration. All values are μU/ml.

that chlorpropamide may *reduce* the plasma response to arginine: five of seven subjects showed a lesser response to arginine after chlorpropamide (Fig. 3).

HGH (Table 4, Fig. 4)

We have previously reported that male subjects show a variable HGH response to arginine infusion (Merimee *et al.*, 1965). This observation was confirmed in the present study: six subjects failed to obtain a rise in plasma HGH (5 ng/ml or above) after arginine, the other subject (L.A.) did (Table 4). No unique pattern emerged after chlorpropamide pre-treatment: that is, the male response remained variable. While two non-responders now showed a good post-arginine rise in plasma HGH, four others did not (Fig. 4).

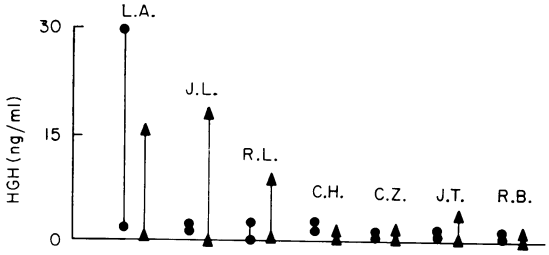

FIG. 4. A comparison of response of plasma HGH concentration to arginine in seven subjects (basal and peak HGH levels shown) without pre-treatment (●) and after chlorpropamide pre-treatment (▲).

Discussion

Fajans *et al.* (1963) were able to increase insulin release after leucine in normal subjects by sulphonylurea pre-treatment. By contrast, we were unable to enhance the arginine-dischargeable insulin pool by pre-treatment with chlorpropamide. We chose male subjects for this study because, in our hands, the increment in plasma insulin concentration after a standard load of arginine monochloride (30 g) in males as a group is comparable to that observed by Floyd *et al.* (1966) after leucine. These circumstances then lend themselves nicely to examination for any augmentation in insulin responsiveness, an observation which might be obscured if the initial plasma insulin responses were already substantial. Sulphonylurea pre-treatment effected no significant change in insulin responsiveness to arginine challenge: mean plasma insulin levels rose from 18·3 to 43·1 μU/ml initially and from 26·1 to 48·6 μU/ml after sulphonylurea pre-treatment. The data were also examined to determine whether the increase in basal plasma insulin levels after chlorpropamide was obscuring any change in responsiveness. The mean increment in plasma insulin after arginine was 25·2 μU/ml before and 22·5 μU/ml after sulphonylurea pre-treatment. Five of the seven subjects exhibited a *decrease* in the arginine-initiated rise in plasma insulin following chlorpropamide pre-treatment (Fig. 3). We conclude, therefore, that sulphonylurea pre-treatment does not enhance arginine-initiated insulin release in normal males, in whom we believed there was the greatest chance of detecting any augmentation. Our findings appear to be similar to the experiments already reported in abstract form by Floyd *et al.* (1965).

It is possible that leucine and arginine provoke insulin release by different mechanisms. It may be useful, therefore, to explore whether insulin release initiated by these two amino acids differs in ways other than the disparate response to sulphonylurea pre-treatment. For example, we have shown that

TABLE 4
Plasma HGH concentration after arginine infusion*

Subject	Control								After chlorpropamide pre-treatment							
Time (min):	−15	0	15	30	45	60	90	120	−15	0	15	30	45	60	90	120
L.A.	2·5	2·5	—	7·5	—	9·0	12·5	30·0	1·5	0	1·5	6·0	16·0	15·5	6·0	2·0
J.L.	1·5	1·5	0·5	2·5	0	1·0	0·5	0	0	0	0	5·0	16·5	18·0	—	0
R.L.	0	0	1·5	0	0·5	3·0	0	0	0·5	0	2·0	1·0	6·5	9·0	2·5	0
C.H.	1·5	1·5	1·5	1·5	2·5	3·0	—	1·5	0	0	0	1·0	1·5	1·0	0·5	0·5
C.Z.	0·5	0·5	0·5	1·5	1·5	1·5	1·5	2·5	0·5	0·5	0·5	0	0·5	0·5	0·5	0·5
J.T.	1·0	0·5	1·0	0·5	1·0	1·0	1·0	1·0	1·5	0·5	0	4·0	1·5	1·5	0·5	1·5
R.B.	1·0	1·0	1·0	1·0	1·0	1·0	1·5	1·5	0	0	0	0	0	0	0	0

* Arginine monochloride refused from time zero to time 30. All values are ng/ml.

epinephrine, which blunts insulin release after intravenous glucose, failed to inhibit arginine-initiated insulin release (Rabinowitz *et al.*, 1966a, b). Also, Fajans *et al.* (1966) have evidence that diazoxide may reduce insulin release after leucine. It will be of interest to determine the influence of epinephrine on leucine-initiated insulin release and of diazoxide on arginine-initiated hormonal release.

We next turn our attention to arginine-initiated HGH release in male subjects. We have previously reported (Merimee *et al.*, 1966) that male subjects show a variable rise in plasma HGH concentration after the infusion of this amino acid: at least half the male subjects we have examined are 'non-responders' (defined as failure of plasma HGH concentration to rise above 5 ng/ml). However, male subjects pre-treated with stilbestrol (2·5 mg b.i.d. for 2 days) consistently display an increment in plasma HGH levels after arginine. In the present group of seven males only one (L.A.) had a convincing rise in plasma HGH after arginine on initial testing, thus confirming the heterogeneity of response. Chlorpropamide pre-treatment failed to produce any consistent effect on arginine-initiated HGH release. The one primary responder (L.A.) again showed a good rise in plasma HGH concentration after arginine; two non-responders now displayed increments in plasma HGH, and the other four subjects remained non-responders. It is important to emphasize that failure to observe a rise in plasma HGH concentration after arginine in the adult male cannot be construed as evidence of HGH deficiency. Subject L.A. has been re-challenged with arginine after stilboestrol pre-treatment and showed a good HGH response; subject R.L. was rendered hypoglycaemic by intravenous insulin administration, and displayed a rise in plasma HGH concentration.

Summary

Male subjects exhibit only a modest increment in plasma insulin concentration after a standard intravenous infusion of 30 g of arginine monochloride and any augmentation of this response should thus be readily detectable. Chlorpropamide pre-treatment in seven male subjects failed to increase the pancreatic islet response to arginine infusion, insofar as this is reflected by peripheral plasma insulin concentrations. Indeed, in five subjects the post-chlorpropamide plasma insulin response to arginine was *less* than the response without pre-treatment. Male subjects show a variable HGH response to arginine infusion. This response remained heterogeneous after chlorpropamide pre-treatment.

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References

COORE, H.G. & RANDLE, P.J. (1964) Regulation of insulin secretion studied with pieces of rabbit pancreas incubated in vitro. *Biochem. J.* **93,** 66.

FAJANS, S.S., FLOYD, J.C., JR, KNOPF, R.F., RULL, J., GUNTSCHE, E.M. & CONN, J.W. (1966) Benzothiadiazine suppression of insulin release from normal and abnormal islet tissue in man. *J. clin. Invest.* **45,** 481.

FAJANS, S.S., KNOPF, R.F., FLOYD, J.C., JR, POWER, L. & CONN, J.W. (1963) The experimental induction in man of sensitivity to leucine hypoglycemia. *J. clin. Invest.* **42,** 216.

FLOYD, J.C., JR, FAJANS, S.S., CONN, J.W., KNOPF, R.F., GUNTSCHE, E. & RULL, J. (1965) Amino acid-induced hyperinsulinemia—Chlorpropamide enhancement only with leucine. *J. Lab. clin. Med.* **66,** 870.

FLOYD, J.C., JR, FAJANS, S.S., CONN, J.W., KNOPF, R.F. & RULL, J. (1966) Stimulation of insulin secretion by amino acids. *J. clin. Invest.* **45,** 1487.

FLOYD, J.C., JR, FAJANS, S.S., KNOPF, R.F. & CONN, J.W. (1963) Evidence that insulin release is the mechanism for experimentally induced leucine hypoglycemia in man. *J. clin. Invest.* **42,** 1714.

MERIMEE, T.J., BURGESS, J.A. & RABINOWITZ, D. (1966) Sex-determined variation in serum insulin and growth hormone response to amino acid stimulation. *J. clin. Endocr.* **26,** 791.

MERIMEE, T.J., LILLICRAP, D.A. & RABINOWITZ, D. (1965) Effect of arginine on serum levels of human growth hormone. *Lancet*, **ii,** 668.

RABINOWITZ, D., MERIMEE, T.J., BURGESS, J.A. & RIGGS, L. (1966a) Growth hormone and insulin release after arginine: Indifference to hyperglycemia and epinephrine. *J. clin. Endocr.* **26,** 1170.

RABINOWITZ, D., MERIMEE, T.J., MAFFEZZOLI, R. & BURGESS, J.A. (1966b) Patterns of hormonal release after glucose, protein, and glucose plus protein. *Lancet*, **ii,** 454.

ROTH, J., GLICK, S.M., YALOW, R.S. & BERSON, S.A. (1963) Secretion of human growth hormone: physiologic and experimental modifications. *Metabolism*, **12,** 577.

YALOW, R.S. & BERSON, S.A. (1960) Immunoassay of endogenous plasma insulin in man. *J. clin. Invest.* **39,** 1157.

YALOW, R.S., BLACK, H., VILLAZON, M. & BERSON, S.A. (1960) Comparison of plasma insulin levels following administration of tolbutamide and glucose. *Diabetes*, **9,** 356.