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THE EFFECT OF HUMAN PLASMA ON THE GLUCOSE UPTAKE OF THE RAT DIAPHRAGM BEFORE AND AFTER ADMINISTRATION OF CARBUTAMIDE

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The glucose uptake of the rat diaphragm has been determined in the presence of the plasma of young volunteers before and after administration of carbutamide without and with added insulin. The increase in the glucose uptake of the rat diaphragm due to the added plasma above that in the medium alone has been termed plasma effect. The increase in the glucose uptake of the rat diaphragm with plasma and added insulin above that with only insulin in the medium has been termed plasma + insulin effect. There was a significant increase in the plasma effect and the plasma + insulin effect after carbutamide administration. The increase in the plasma + insulin effect was significantly greater than the increase in the plasma effect. From these observations it has been suggested that carbutamide potentiates the action of insulin peripherally. Observed facts about carbutamide do not contradict this mechanism of action.

Since the articles published in 1955 about the oral anti-diabetic drug, carbutamide (BZ-55), work has been carried out at various places to confirm its hypoglycaemic property and to study the mechanism of its action. The former action is now fairly well established and the drug has been recommended in certain types of diabetes mellitus.

The mechanism of the hypoglycaemic action is, however, uncertain. Originally from the histological studies of the pancreas, it was suggested that BZ-55 caused structural destruction (Frank and Fuchs, 1956) or functional inhibition of the α cells. Other observers failed to confirm this (Ferner and Runge, 1956). Mirsky, Perisutti, and Diengott (1956) suggested that BZ-55 inhibited the insulin destroying system (insulinase). Vaughan (1956) could not confirm this. She as well as others (Hawkins, Ashworth, and Haist, 1956; Tyberheim, Hasley, and Williams, 1956) showed that BZ-55 prevented glycogenolysis in the liver. Young (1956) introduced a novel idea by proposing that this and the other hypoglycaemic substances destroyed the organisms in the pool at the bottom of the portal drain which might be responsible for the destruction of insulin. But he himself has doubted this, as another hypoglycaemic compound, tolbutamide (D860, Orinase), is devoid of antibacterial activity. Loubatieres (1955), Ashworth and Haist (1956), and Pozza, Galansino, and Foa (1956) maintain that BZ-55 stimulates insulin secretion.

Gemmill (1940) first showed that insulin increases glycogen synthesis by the isolated rat diaphragm. Recently Randle (1954) and Vallance-Owen, Hurlock and Please (1955) have studied the effect of plasma on the glucose uptake of the rat diaphragm as a means of estimating plasma insulin levels. The experiments reported here are concerned with the effect of plasma on the glucose uptake of the isolated rat diaphragm before and after administration of BZ-55 and also with the effect of such plasma with added insulin.

METHODS AND MATERIAL

Ten housemen of Sassoon Hospital, Poona, aged 23 to 28 years, acted as subjects for this work. Each volunteer fasted from 10 p.m. on the previous night. Ten ml. of venous blood was collected in a heparinized syringe at 8 a.m. One ml. of this blood was used for blood sugar estimation, which was made by the Folin method. The remaining blood was centrifuged to separate the plasma, which was stored in a refrigerator but was always used within one hour of its withdrawal.

White rats, weighing between 120 to 180 g., were used. Each rat was starved for 24 hr., killed by stunning, and the two hemi-diaphragms removed quickly with as little trauma to the tissue as possible. Each hemi-diaphragm was bisected and each of the four quarter diaphragms was incubated in one of the following: (I) medium; (II) plasma-medium mixture; (III) plasma-medium-insulin mixture; (IV) insulin-medium mixture. The Stadie and Zapp (1947) phosphate buffer (*p*H 6.8) containing 0.2%

glucose was used as the medium. The plasma-medium mixture contained 2 ml. plasma + 2 ml. medium. The plasma-medium mixtures were prepared as directed by Randle (1954), but the proportions were different. The plasma-medium-insulin mixture was plasma-medium mixture containing 10^{-3} units of insulin/ml. The insulin-medium mixture was medium containing 10^{-3} units of insulin/ml. It was found by direct measurement that the glucose concentration in the plasma-medium mixture did not fall below 0.15%. The volume of incubation fluid was 2 ml. in each case.

Incubation was carried out in the Dubnoff metabolic shaking incubator at 38° with 100% oxygen as gas phase for a period of 90 min. with a shaking rate of 112 oscillations/min. Simultaneously, four similar vessels containing 2 ml. each of the above incubation fluids but without the diaphragms were incubated as controls. At the end of the incubation period the diaphragms were weighed, and the glucose in the control and the incubated samples estimated by the Folin micromethod. The difference between the control and the incubated samples was taken as the glucose uptake by the rat diaphragm, which was calculated as mg. glucose/g. wet weight of the diaphragm/hr. The difference between (II) and (I) was taken as the plasma effect (Table II) and the difference between (III) and (IV) was taken as the plasma+insulin effect. (I) and (II) did not contain any added insulin. Here the increase in (II) over (I) is due to the plasma when the insulin concentration in the medium was zero. (III) and (IV) contained insulin in the concentration of 10^{-3} units of insulin/ml. The increase in (IV) over (I) was due to the added insulin. This increase must also be present in (III). But (III) contains plasma in addition. Thus increase in (III) over (IV) is due to the plasma over the basal concentration of insulin, which was 10^{-3} units/ml. This was termed as plasma+insulin effect, by which is meant the effect of plasma alone in the presence of added insulin.

On the day the first blood sample was taken each volunteer received 1 g. of carbutamide (Invernol, Hoechst-Fedco Pharma) on two occasions. Thereafter, 0.5 g. was given three times a day on the subsequent three days; 0.5 g. on the morning of the fourth day. A fasting sample of blood was collected 2 hr. later and treated in the same way as the previous one. Each volunteer thus received a total of 5.5 g. of BZ-55 in 68 hr.

Results

All the volunteers remarked on the increase in their appetite on the third and the fourth days. Most of them complained of dizziness and cramps in their legs. A few of them complained of hypotonia and mental excitement. Two complained of tremors and unsteadiness on the morning of the fourth day. Symptoms were aggravated when meals were delayed and relieved after taking food. The symptoms persisted for a day after withdrawal of the drug and in one case for two days. Column (c) of Table I gives the fall in the fasting blood sugar after taking the drug. The mean of the fall was 42.7 mg.%. None of the volunteers developed hypoglycaemic shock.

TABLE I

FASTING BLOOD SUGAR OF NORMAL MEN BEFORE AND AFTER ADMINISTRATION OF BZ-55

No. of Man	Fasting Blood Sugar (mg.%)		
	Before BZ-55 (a)	After BZ-55 (b)	Fall after BZ-55 (c)=(a)−(b)
1	106	72	34
2	98	66	32
3	90	48	42
4	100	41	59
5	114	38	76
6	105	64	41
7	84	56	28
8	90	60	30
9	102	66	36
10	107	58	49
			Mean 42·7

In Table II, cols. (a) and (b) give the plasma effect before and after administration of BZ-55 respectively. Column (c) gives the increase in the plasma effect ((b)−(a)) after BZ-55 administration. Columns (d) and (e) give the plasma+insulin effect before and after administration of BZ-55 respectively, and col. (f) gives the increase in it ((e)−(d)) after BZ-55.

The mean plasma effect and the mean plasma+insulin effect before BZ-55 administration were 0.689 and 0.639 respectively with practically no difference between the two. After BZ-55 administration the mean values of these effects were 1.130

TABLE II

PLASMA EFFECT AND PLASMA+INSULIN EFFECT BEFORE AND AFTER ADMINISTRATION OF CARBUTAMIDE (BZ-55)

Tissue, isolated rat diaphragm muscle. Plasma effect=Glucose uptake in [plasma+medium]−glucose uptake in the medium. Plasma+insulin effect=Glucose uptake in [plasma+medium+insulin]−glucose uptake in [medium+insulin]. Insulin concentration 10^{-3} units/ml. in each experiment. Medium=The Stadie and Zapp (1947) phosphate buffer (*p*H 6.8) containing 0.2% glucose. For further explanation see text.

No. of Man	Plasma Effect (mg./g./hr.)			Plasma+Insulin Effect (mg./g./hr.)		
	Before BZ-55 (a)	After BZ-55 (b)	Increase After BZ-55 (c)	Before BZ-55 (d)	After BZ-55 (e)	Increase After BZ-55 (f)
1	0·20	1·20	1·00	0·20	2·80	2·60
2	1·10	0·56	−0·54	0·40	1·20	0·80
3	0·80	1·80	1·00	0·20	1·50	1·30
4	1·29	2·27	0·98	0·80	0·32	−0·48
5	0·40	0·52	0·12	0·05	2·05	2·00
6	0·75	0·94	0·19	1·23	2·56	1·33
7	0·70	1·16	0·46	1·16	1·86	0·70
8	0·86	1·53	0·67	1·30	2·20	0·90
9	0·33	0·42	0·09	0·45	1·28	0·83
10	0·46	0·90	0·44	0·60	2·28	1·68
Mean	0·689	1·130	0·441	0·639	1·805	1·166

and 1.805 respectively. The plasma + insulin effect is significantly greater than plasma effect after BZ-55 administration ($t=2.24$, $P<0.05$). The absence of any difference between the two values before BZ-55 administration suggests that the starting point on the dose/response curve does not significantly affect the increase in the glucose uptake due to the plasma. Hence the greater increase in the plasma + insulin effect seen after BZ-55 administration is unlikely to be due to this factor. The mean increase in the plasma effect after BZ-55 administration was 0.441. The mean increase in the plasma + insulin effect after BZ-55 administration was 1.166, which is significantly greater than the increase in the plasma effect ($t=2.34$, $P<0.05$). Our results thus show a fall in the fasting blood sugar, an increase in the plasma effect and an increase in the plasma + insulin effect. The increase in the plasma + insulin effect is significantly greater than the increase in the plasma effect.

Discussion

Field and Woodson (1956) have reported that the increase in glycogen synthesis of rat diaphragm due to insulin was depressed by BZ-55 and sulphadiazine in concentrations of 2.0 mg./ml. The insulin effect was not affected by BZ-55 in a concentration of 0.2 mg./ml. Greater concentrations of BZ-55 without insulin did not affect glycogen synthesis of the rat diaphragm. Cahill, Hastings, and Ashmore (1957) have reported that BZ-55 and tolbutamide did not alter the insulin effect on glucose uptake of the rat diaphragm in a concentration of 50 mg.% with insulin concentrations ranging between 0.25 milliunits to 25 milliunits/ml. Clarke, Davidson, Schonbaum and Senman (1956) reported that BZ-55 in a concentration of 6.0 mg.% caused depression of glucose uptake of the rat diaphragm. Addition of insulin overcame this depression and produced the usual increase in the glucose uptake, thus giving an apparent increase in the insulin effect. Though the authors do not think that this was due to increased glucose uptake in presence of BZ-55 + insulin, they do not rule out such a possibility.

We used plasma containing BZ-55 to test the effect on the glucose uptake of the rat diaphragm. Hence the drug was present in the form in which it circulates in the plasma and in the concentration in which it caused hypoglycaemia in the volunteers. From the results presented in the Tables it is obvious that the insulin-like effect of the plasma increased after the drug administration. Our finding that the increase in the plasma + insulin effect was significantly greater than the increase in the plasma effect is perhaps more important. There was practically no difference between the plasma effect and the plasma + insulin effect before administration of BZ-55. After BZ-55 administration the plasma contained free sulphonamide; the plasma also contained insulin; and an increase in the plasma effect after BZ-55 administration might be explained by assuming that BZ-55 in the plasma enhanced the action of whatever insulin was present there. In the case of plasma + insulin effect there was a greater amount of insulin in the medium and hence the increase in the plasma + insulin effect is greater than the increase in the plasma effect. Hence our results suggest that BZ-55 potentiates the peripheral action of insulin.

If the increase in the plasma effect and the increase in the plasma + insulin effect were due to the direct action of the drug present in the plasma, then the increase in the plasma effect should not have been different from the increase in the plasma + insulin effect, because the same amount of the drug was added to test both effects, namely that present in 1 ml. of plasma. On the contrary, we obtained a significantly greater increase in the plasma + insulin effect.

Similarly if we assume that BZ-55 increases the insulin concentration of the plasma by either stimulating its secretion (Ashworth and Haist, 1956; Loubatieres, 1955) or preventing its destruction (Mirsky, 1956), then we would not have expected to obtain the statistically significant difference between the plasma effect and the plasma + insulin effect after administration of BZ-55 which we found (Table II, cols. b and e).

Our assumption that BZ-55 potentiates the peripheral action of insulin is not incompatible with the observed facts about BZ-55 action.

It has been observed that BZ-55 lowers the blood sugar to a certain level. In rabbits if the dose is still further increased hyperglycaemia results (Achelis and Hardebeck, 1956). When a man commences taking the drug, it will cause a fall in the blood sugar as it will enhance the action of insulin that is present in the plasma. As the blood sugar falls the insulin secretion will decrease. This decrease will progress until, at a certain degree of hypoglycaemia, insulin release ceases and no further fall in the blood sugar will occur. As the insulin disappears from the plasma, there will be rapid glycogenolysis and hyperglycaemia will result.

Failure of the drug in diabetes mellitus cases with very high blood sugar is to be expected

because in these cases very little insulin, if any, is present in the plasma (Vallance-Owen *et al.*, 1955). It then follows that BZ-55 should be most effective in cases with relative insulin deficiency. It is an accepted fact that cases suitable for BZ-55 therapy are those of obese, mild diabetics at or over middle age. When a patient receives insulin regularly over a prolonged period of time, it is possible that secretion of insulin in such a patient is depressed as happens with most of the other hormones. This would explain the failure of BZ-55 in cases which have received prolonged insulin therapy. Our view is strengthened by the observation that BZ-55 when given alone does not cause hypoglycaemic shock, but, after its administration with smaller doses of insulin, hypoglycaemic shock may arise (Bertram, Benfeldt, and Otto, 1956).

The tendency of BZ-55 to cumulate should be borne in mind in its clinical use. Lowering of the blood sugar below the physiological limits may convert a relative insulin deficiency into an absolute one by prolonged depression of β cells. This can be avoided by reducing the maintenance dose of the drug to the minimum and ensuring that the fasting blood sugar does not fall below normal limits.

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