

ADRENERGIC BLOCKING ACTION OF CARBUTAMIDE ON THE PERIPHERAL UTILIZATION OF GLUCOSE

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Effect of epinephrine on the increment index calculated from intravenous glucose tolerance test before and after carbutamide therapy is studied in normal and alloxan diabetic dogs. Carbutamide in itself is found to have no action on peripheral utilization of glucose though the reduction in peripheral utilization of glucose due to epinephrine action is blocked completely in diabetic dogs and to a certain extent in normal ones.

Even after the establishment of the therapeutic effectiveness of the sulphonylureas in the treatment of certain types of diabetes mellitus, the exact mechanism of its action is still a matter of conjecture. A chance observation by a French physician Janbon that sulphonamide causes a fall in blood-sugar and later on the ingenious work by Loubatieres during the period 1942-1952 paved the way for the discovery of the more potent and less toxic sulphonylureas. Loubatieres (1955) proposed that the compound 2254 RP (IPTD) produces the hypoglycaemic effect due to β -cell stimulation. Similar mechanism has been proposed for the sulphonylureas, carbutamide and tolbutamide (Ashworth and Haist 1956, Pozza, 1956; Root, 1957) various other mechanisms have been postulated such as (i) potentiation of insulin action (Sirek and Sirek, 1956; Cambell, 1956; Aiman and Kulkarni; 1957), (ii) inhibition of liver glycogenolysis (Purnell 1956, Kibler and Gordon 1956; Anderson *et al.*, 1956; Tyberhein *et al.*, 1956; Hawkins *et al.*, 1956), (iii) direct insulin like action on tissues (Lundback Nielson, 1958), (iv) liberation of bound insulin (Aiman and Chaudhary, 1959), (v) inhibition or damage of α -cells of Langerhans (Holt *et al.*, 1955; Frank and Fuchs 1956) and (vi) anti-insulinase action (Mirsky *et al.*, 1956 a, 1956b).

Epinephrine induces hyperglycaemia due to its dual action on carbohydrate metabolism. It causes increased liver glycogenolysis and reduction in the peripheral utilization of glucose. Present study is undertaken to see if sulphonylureas have any blocking action on the above mentioned peripheral effect of epinephrine on the glucose utilization, and whether that can be a possible mechanism of its action.

Various methods *in vivo* and *in vitro* are available for studying the glucose utilization of tissues and drug action on it. The methods useful in intact animal are (i) estimation of basal non-protein respiratory quotient (Chambers, 1935), (ii) arterio-venous glucose concentration differences: calculation of the assimilation index and relative assimilation index there from (Somogyi, 1950; Elrick and Hlad 1955) or plotting the 'A-V' area (Somogyi, 1951), (iii) intravenous glucose tolerance test and calculation of the increment index (Greville, 1943; Amatuzio *et al.*, 1953; Duncan, 1956), and (iv) radio-isotope dilution technics using radioactive glucose (Steele *et al.*, 1956).

In vitro methods are measurement of glucose uptake (i) in hindlimb preparation (Griffith *et al.*, 1949) and (ii) in isolated tissues such as rat diaphragm or any muscle or liver slices, etc.

The method that is used in the present study is the intravenous glucose tolerance test and calculating the increment index. Besides being easy, quick and safe this test has an added advantage. Since glucose is administered intravenously, no question of delay in absorption is encountered. After the glucose load is administered, by studying the subsequent blood sugar levels one can quantitatively obtain the value by which the glucose is eliminated from the body. The increment index is calculated which expresses the rate of fall of glucose level per min as a percentage of level above fasting value (glucose increment).

METHOD

The intravenous glucose tolerance test was done on male dogs, four normal and six alloxan diabetic. Alloxan diabetes was induced by administration of alloxan monohydrate (intravenously 60 mg/kg). Blood-sugar and urine examination for sugar was studied on these dogs for the establishment of the diabetic state. The animal was used about a week after the alloxan administration. Two out of the six dogs had diabetes established previously. These dogs were stabilised and maintained on insulin injection. In these two dogs, insulin injections were omitted seventytwo hours prior to the commencement of this study.

On each animal four intravenous glucose tolerance tests were done; (i) first day control test. (ii) On third day - 30 min after injection of 0.5 mg epinephrine subcutaneously. Carbutamide 100 mg/kg/day as a suspension in water orally was commenced immediately after (ii) and continued for 7 days. (iii) On seventh day of therapy. (iv) Next day as in (ii) i.e., 30 mins after injection of epinephrine 0.5 mg subcutaneously.

The animal was fasted overnight for 18 hrs. Paraldehyde 1 ml/kg was administered by intragastric tube for sedation. This was necessary to exclude the possible effect of endogenous epinephrine release due to excitation during taking the blood samples. After the animal was well sedated, fasting blood sample was taken. Glucose as 50 per cent solution in dose of 1 g/kg was administered intravenously in 2-3 mins. Three minutes after completion of the glucose injection, the blood sample was taken (zero time) so as to give the maximum increment attained over the fasting blood sugar. Subsequently, the blood samples were taken at 10 mins intervals for 1 hr.

Blood sugar estimations were done by micro-method of Folin and Malmaros (1929).

Calculation of the increment index. The increment over the fasting blood sugar level was calculated for each sample by deducting the fasting blood sugar value from the value at a particular interval. By applying the analytical method (Greville 1943; Amatuzio *et al.*, 1953; Duncan, 1956) the increment index was calculated using the formula:—

$$K = 2.3 \times \frac{\text{Log}_{10} C_1 - \text{Log}_{10} C_2}{t_2 - t_1}$$

Where K = the increment index which expresses the rate of fall of glucose level per min as a percentage of level above the fasting level. C_1 = increment in mg above fasting blood sugar at time t_1 . C_2 = increment in mg above fasting blood sugar at time t_2 . $t_2 - t_1 = 10$ min as the blood samples were collected at 10 min interval.

RESULTS

From Tables I and II it will be seen that (i) peripheral utilization of glucose is markedly depressed in diabetic state; (ii) epinephrine diminishes the peripheral utilization of glucose in both the normal as well as in alloxan diabetic dogs; (iii) carbutamide has no effect on peripheral utilization of glucose in both normal and in alloxan diabetic dogs; (iv) after carbutamide therapy, the peripheral depressant effect of epinephrine on the glucose utilization is partially blocked in normal dogs, whereas it is completely blocked in alloxan diabetic dogs. The results, therefore, would indicate that carbutamide while not itself affecting the peripheral utilization of glucose, blocks the epinephrine depressant effect.

TABLE I

Mean increment indices in normal dogs

Dog	I. V. test Control	I. V. test after 0.5 mg epinephrine	Reduction in index due to epinephrine action	Carbutamide therapy - 7 days 100 mg/kg daily as aqueous solution, orally	I. V. test Control after therapy	I. V. test after 0.5 mg epinephrine	Reduction in index due to epinephrine action
	1	2	3		4	5	6
X	7.2	4.3	2.9		6.7	5.8	0.9
N	6.5	4.3	2.2		6.4	5.7	0.7
B	7.0	4.0	3.0		6.1	5.1	1.0
Z	6.1	3.8	2.3		6.2	5.3	0.9
Mean	6.7	4.1	2.6		6.35	5.47	0.9
±SE of Mean	±0.25	±0.125	±0.2		±0.135	±0.165	±0.065

- (i) Epinephrine depresses the mean increment index (Columns 1 and 2) significantly ($t=10$; $P<0.01$).
- (ii) Carbutamide therapy produced no significant change in the index (Columns 1 and 4) ($t=1.4$; $P>0.10$)
- (iii) Epinephrine even after carbutamide therapy depressed the mean increment index (Columns 4 and 5) ($t=4.02$; $P<0.01$).
- (iv) Reduction in the mean increment index due to epinephrine action was much less after carbutamide therapy (Columns 3 and 6) ($t=7.4$; $P<0.01$)

DISCUSSION

The test, though has many advantages, suffers from a serious drawback namely, it is unphysiological. The glucose load, subjected to the body at one time, may upset the homeostatic mechanisms. Thus the above results should be evaluated with certain reservations in mind.

Another possible objection would be that after the glucose is administered intravenously, the blood glucose level at zero-time crosses the renal sugar threshold and one must expect that some amount of glucose will be lost in urine, much more so in case of diabetics where the initial fasting blood sugar

sialready at a higher level. If urinary loss of glucose would have been the only difference in glucose utilization in the normal and in the diabetic states, since the urinary loss is marked in diabetes, the increment index should have increased in diabetics than in normal. But the increment index in diabetic dogs is definitely lowered than in normal dogs, the results being statistically significant Table II. The only explanation for this reduction of index is marked reduction in peripheral utilization of glucose in diabetes. Other workers

TABLE II

Mean increment indices in diabetic dogs

Dog	I. V. test Control	I. V. test after 0.5 mg epinephrine	Reduction in index due to epinephrine	Carbutamide therapy - 7 days 100 mg/kg daily as aqueous suspension, orally	I. V. test Control after therapy	I. V. test after 0.5 mg epinephrine	Reduction in index due to epinephrine action
	a	b	c		d	e	f
H	1.35	0.76	0.59		1.50	1.25	0.25
J	2.65	1.36	1.29		2.87	2.28	0.59
N	1.40	0.80	0.60		1.52	1.33	0.19
Z	1.33	0.77	0.56		1.48	1.24	0.24
A	1.32	0.79	0.53		1.42	1.18	0.24
D	1.40	0.73	0.67		1.41	1.34	0.07
Mean	1.57	0.87	0.70		1.7	1.44	0.26
±SE of Mean	±0.21	±0.1	±0.11		±0.232	±0.17	±0.07

- (i) The mean increment index is markedly depressed in diabetic dogs than that in normal dogs (Column a and 1 Table I) ($t=15; P<0.01$)
- (ii) Epinephrine depresses the peripheral utilization in diabetic dogs still further (Columns a and b) ($t=18; P<0.01$).
- (iii) Carbutamide therapy produced no significant change in the index (Columns a and d) ($t=3; P>0.01$)
- iv) Epinephrine after carbutamide therapy, produced no significant depression in index (Columns d and e) ($t=0.9; P>0.10$).

(Amatuzio *et al.*, 1953; Duncan, 1956) have obtained similar reduction in increment index for diabetic human beings. Jokipii and Turpeinen (1954) have mentioned that the fall in glucose level gives the combined picture of peripheral utilization of glucose and the urinary loss of glucose especially in diabetics. If urinary loss is considered as a separate entity during calculation of the index and if this loss is deducted from the total fall of glucose in 10 mins, the increment index will be much more reduced. In calculations done for this study urinary loss of glucose was not determined since this elimination of glucose in diabetics was acting in an opposite direction and did not viciate the results.

No reports in literature are available on intravenous glucose tolerance in experimental animals. Most of the reported work is on human beings in health and disease. Amatuzio *et al.*, (1953) and Duncan (1956) have reported the range of increment indices obtained in normal and mild diabetic human beings. The values of the indices are 2.97 to 4.85 (Amatuzio *et al.*, 1953) and 3.15 to 4.62 (Duncan, 1956) in normal human beings and 0.93-2.46 (Amatuzio *et al.*, 1953) and (Duncan, 1956) in mild diabetic human beings. Amatuzio *et al.*, (1953) obtained the figures of 0.19-1.64 in case of severe diabetics.

In a small series as reported herein on dogs, the values for the mean increment indices are 6.7 ± 0.25 in normal dogs and 1.57 ± 0.21 in diabetic dogs. The marked reduction in the increment index in diabetic state is quite in accordance with the results obtained in human beings.

Butterfield *et al.*, (1957) and Bastenie *et al.*, (1957) have carried out intravenous glucose tolerance test on human diabetics after treating them with carbutamide. They observed no change in the glucose assimilation index.

In this work, carbutamide therapy did not show any significant alteration of the increment index both in normal and in diabetic dogs. (Table I and II).

The effects of epinephrine on carbohydrate metabolism have been very intensively studied by many workers including Somogyi (1950; 1951). It has been convincingly shown that the depressant effect of epinephrine on peripheral utilization of glucose by calculating relative assimilation index or plotting the 'A-V' area. In his opinion the increased amount of glucose detected in hepatic outflow after an injection of epinephrine is also due to depressed uptake of glucose by the liver cells.

Walaas and Walaas (1950) have similarly shown diminished uptake of glucose by rat diaphragm after addition of epinephrine.

Amatuzio *et al.*, (1954) observed marked reduction in the increment index, while studying the effect of epinephrine on intravenous glucose tolerance test on normal human beings. Both on normal and diabetic dogs similar depressant effect of epinephrine on the peripheral utilization of glucose has been observed in the present study.

Certain workers like Griffith *et al.*, (1949) and Ingle and Nezamis (1949) have observed increased uptake of glucose after epinephrine which has not been confirmed in this work on dogs.

Epinephrine also causes increase in the rate of hepatic glycogenolysis (Ellis and Anderson 1951; Bloom and Russel, 1955; Bearn *et al.*, 1951). It has been noted that the peak glycogenolytic effect of epinephrine is reached in about 25-30 mins (Amatuzio *et al.*, 1954; Somogyi, 1951). Since the intravenous glucose tolerance test was carried out 30 mins after the administration of epinephrine, the maximum glycogenolytic effect was expected to be reached prior to the commencement of the test. Amatuzio *et al.*, (1953) and Duncan (1956) have shown in human beings that the increment index is a constant index independent of the glucose load. Thus the hepatic effects of epinephrine do not come into the picture while the calculations for the increment index are done. The increment index, as determined from the intravenous glucose tolerance test after epinephrine injection, thus shows only the effects of epinephrine on the peripheral utilization of glucose.

Moorehouse and Kark (1956) studied the effect of orinase (D-860) on epinephrine response in healthy young men. The rate and the extent of the response of the blood glucose level to epinephrine was unaltered. They have suggested a normal spontaneous epinephrine response occurring in a diabetic patient during hypoglycaemia.

Fajans *et al.* (1956) have observed that the hyperglycaemic effect of intramuscularly administered epinephrine is not blocked in normal males by administration of carbutamide or tolbutamide.

Heineman *et al.* (1956) has noted that the hyperglycaemic effect of a test dose of epinephrine is not diminished by the chronic administration of the sulphonylureas to diabetic patients. Cox and Henley (1956) have confirmed that there is no change in epinephrine response after sulphonylurea therapy in diabetic human beings.

However, *in vitro* studies on rat and rabbit liver slices Vaughan (1956, 1957) has shown definite inhibition of the glycogenolytic effect of epinephrine after tolbutamide. Berthet *et al.* (1956) have confirmed inhibition of epinephrine response in dog and rabbit liver slices in presence of D-860 (tolbutamide). This inhibition of epinephrine response by tolbutamide observed in liver slices has been explained by Field and Woodson (1956) by postulating a possible nonspecific poisoning effect of tolbutamide on several tissue enzymes.

Thus the data so far available in literature as to the effect of the sulphonylureas on the epinephrine response are conflicting and no attempt has been made to study the drug action on the epinephrine effect on peripheral utilization of glucose. After carbutamide therapy, the reduction in index due to epinephrine action was not observed in the diabetic dogs. In normal dogs, though reduction in index after epinephrine was noted even after carbutamide therapy, it was much less than before the therapy. This shows that the depressant effect of epinephrine on the peripheral utilization has been completely blocked by carbutamide in diabetic dogs. In normal dogs, however, only a partial block was noted.

From the set up of the present study the effect of carbutamide on the peripheral effects of epinephrine can only be studied. A different experimental set up will have to be designed to study the action of carbutamide on hepatic glycogenolytic effect of epinephrine.

In short, from the present study it can be surmised that the depressant effect of epinephrine on peripheral utilization of glucose is blocked by carbutamide completely in diabetic dogs. But since the hyperglycaemia observed in diabetes is not due to epinephrine action, this possible mechanism of action of carbutamide plays only an insignificant role in control of diabetes. Carbutamide therapy according to this mode of action can only block the peripheral depressant effect of endogenously released epinephrine, the hepatic effects of the same have not been studied in this present work.

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