Brit. J. Pharmacol. (1959), **14**, 377.

MECHANISM OF ACTION OF ORAL ANTI-DIABETIC DRUGS

BY

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(RECEIVED MAY 4, 1959)

The fall in blood sugar with carbutamide (BZ55) 24 hr. after the maintenance dose of insulin (28.1% ±2.9 s.e.) in rabbits made severely diabetic with alloxan was significantly different from the change in the blood sugar with the same drug given 72 hr. (6.8% ±2.0) or distilled water given 24 hr. (7.7% ±1.3) respectively after the last dose of insulin. It is postulated that the drug acts by liberating bound insulin in the plasma.

In spite of the widespread use of carbutamide, the mechanism of action of this drug is uncertain. Work done in this laboratory (Aiman and Kulkarni, 1957) showed that carbutamide potentiated the action of insulin on the rat diaphragm. This finding suggested that the drug might act on the binding of insulin with the plasma proteins. Sirek and Best (1956) have shown that the growth hormone of the anterior pituitary may release bound insulin, because it causes a fall in the blood sugar of depancreatized dogs if injected 24 hr. after, but not if injected 72 hr. after, the last maintenance dose of insulin.

This work was undertaken to demonstrate a similar effect of carbutamide on bound exogenous insulin given to severely diabetic rabbits.

METHODS

Six male rabbits weighing between 1 and 2 kg. were used. They received a standard diet fed for a fixed number of hours daily. The rabbits were rendered diabetic by intravenous injections of 180 mg./kg. of body weight of alloxan monohydrate after a fasting period of 48 hr. If the diabetes showed regression, alloxan injections were repeated till the fasting blood sugar was at least 300 mg./100 ml.

After diabetes had been established for eight days with a fasting blood sugar of about 300 mg./100 ml. or more, 1 unit of insulin zinc suspension (Lente) was given daily for a further eight days, during which the fasting blood sugar was maintained between 190 and 280 mg./100 ml.

Then the blood sugar values were studied over a period of 7 hr. hourly except the first and fourth hours in the following manner: (1) On the first day after 50 ml. of distilled water had been given orally; (2) on the fifth and seventh days after 500 mg./kg. of body weight of carbutamide [BZ55, Nadisan (Neo-Pharma Private Ltd., Bombay)] had been given orally, but having omitted the usual insulin injections on the fifth and sixth days. (3) On the third day, only the fasting blood sugar was determined. Insulin was then restarted and continued for 4 days and the blood sugar curves were studied as before, but giving 50 ml. of distilled water 72 hr. after the last dose of insulin.

The method of Folin and Malmros (1932) was used for blood sugar estimations.

RESULTS

The mean blood sugar values are given in Table I. The maximum % fall in blood sugar in each rabbit under the various experimental conditions are given in Table II, in which it can be seen that the difference between the falls after carbutamide and those after distilled water differ significantly ($P > 0.01$). Fig. 1 shows the mean fall in each of the experiments in which the fasting blood sugar was adjusted to equal 100 mg./100 ml.

TABLE I

MEAN BLOOD SUGAR VALUES

Mean blood sugar values ± s.e. of mean. The numerals in parentheses indicate the number of animals used.

Samples	Mean Blood Sugar Values			
	With Distilled Water		With Carbutamide	
	24 Hr. after Insulin (6)	72 Hr. after Insulin (3)	24 Hr. after Insulin (6)	72 Hr. after Insulin (6)
Fasting blood sugar ..	242 ± 13·2	277 ± 11·4	238·5 ± 14·6	294 ± 5·4
After 2 hr.	245 ± 15·3	290 ± 10·9	230·9 ± 16·4	299 ± 8·2
,, 3 ,,	233 ± 13·2	312·6 ± 23·9	206·5 ± 12·9	294·7 ± 10·5
,, 5 ,,	225 ± 12·6	284 ± 13·6	188·5 ± 13·3	285·9 ± 10·5
,, 6 ,,	231 ± 15·5	283 ± 9·1	179·7 ± 13·8	274·8 ± 3·2
,, 7 ,,	236 ± 14·8	299 ± 21·8	185·5 ± 13·9	282·0 ± 5·1

TABLE II

MAXIMUM PERCENTAGE FALL IN THE BLOOD SUGAR UNDER THE VARIOUS EXPERIMENTAL CONDITIONS

The maximum % fall in Col. 1 when compared with that in Col. 3 is significant ($t=6.1$; $P<0.01$). The maximum % fall in Col. 1 when compared with that in Col. 2 is significant ($t=6.2$; $P<0.01$). The maximum % fall in Col. 2 when compared with that in Col. 3 is not significant ($t=0.4$; $P>0.5$).

Rabbit No.	Maximum Percentage Fall in the Blood Sugar (mg./100 ml.)		
	With Carbutamide		With Distilled Water
	24 Hr. after Insulin	72 Hr. after Insulin	24 Hr. after Insulin
	1	2	3
1	38·8	7·9	10·1
2	25·0	9·8	8·6
3	33·2	9·7	7·4
4	22·8	10·0	9·3
5	18·0	7·7	8·5
6	30·6	4·5	2·3
Mean ± s.e. of mean ..	28·1±2·99	6·8±2·0	7·7±1·3

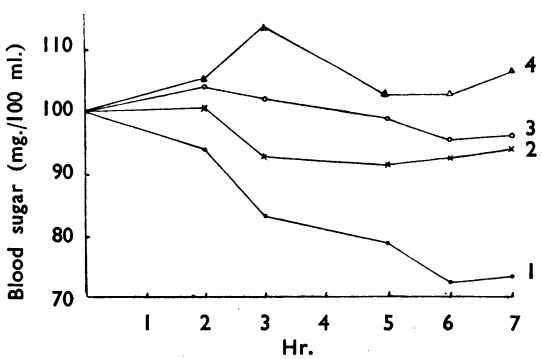

FIG. 1.—Mean falls in blood sugar with carbutamide and distilled water in diabetic rabbits 24 and 72 hr. after the last dose of insulin. The fasting blood sugar was adjusted to 100 mg./100 ml. **1**, with carbutamide 24 hr. after insulin. **2**, with distilled water 24 hr. after insulin. **3**, with carbutamide 72 hr. after insulin. **4**, with distilled water 72 hr. after insulin.

From these results it can be seen that carbutamide given 24 hr. after insulin produced a marked and significant fall in the blood sugar when compared to the fall with the same drug given 72 hr. after insulin.

DISCUSSION

The rabbits were used about three weeks after the alloxan injections when they were severely diabetic as can be seen from the fasting blood sugar estimations in Table I, and hence there was very little chance of endogenous insulin being present in any appreciable quantity; whatever effect was obtained with the drug was therefore due to an action other than through the β cells.

Carbutamide was used in the present study because the mechanisms of action of carbutamide and of tolbutamide (D860) are probably similar and because carbutamide can be administered more easily than can tolbutamide. The effect of the insulin used lasted up to 24 hr. and all experiments were therefore carried out at least 24 hr. after the last dose of insulin.

Recently Randle and Taylor (1958), Marsh *et al.* (1952) and Bornstein (1953) have shown that insulin can be bound to plasma proteins, but the factors determining the unbinding have yet to be clearly demonstrated. Welsh, Henley, Williams, and Cox (1956) have reported that insulin labelled with [^{131}I] disappeared less rapidly from the plasma of diabetic than from that of non-diabetic patients and the binding action of diabetic plasma with insulin reduced its hypoglycaemic action in mice.

In our experiment the fall with carbutamide given 24 hr. after insulin was much more marked than that with carbutamide given 72 hr. after insulin, or that with distilled water given 24 hr. after insulin (Fig. 1). Also the fall with carbutamide given 72 hr. after insulin was not significantly different from that with distilled water given 24 hr. after insulin (Table II). To substantiate our findings, the blood sugar curves were carried out with distilled water given 72 hr. after insulin in three rabbits (Table I). The change in blood sugar in this condition when compared with the change with carbutamide given 72 hr. after insulin showed no significant difference ($t=1.75$, $P>0.1$). Hence it would appear that, since the action of carbutamide was dependent upon the interval after the last dose of insulin, it acted by potentiating insulin in some way.

Our results suggested the possibility that carbutamide produced a significant fall in blood sugar when given 24 hr. after insulin, by influencing any insulin in bound form in a manner similar to that reported by Sirek and Best (1956) with growth hormone in depancreatized dogs. While it is also possible that the exogenous insulin "permitted" the hypoglycaemic effect of a direct action of carbutamide on the liver to reduce its glucose output (Ricketts, Wildberger, and Schmid, 1957), the results obtained in studies in which insulin was added to incubation flasks containing rat diaphragms (Aiman and Kulkarni, 1957) support a potentiating effect rather than a "permissive" liver action. The sulphonylureas are bound to plasma proteins. Is it possible that this binding in some way affects the equilibrium of bound insulin and that the direct pancreatic action (Loubatières, 1955; Pozza *et al.*,

1956; Ashworth *et al.*, 1956) may be an indirect effect resulting from altered plasma insulin levels?

This work was undertaken by N. Chaudhary during the tenure of a Government of Bombay Research Grant

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