THE ROLE OF LIVER IN THE MECHANISM OF HYPOGLYCEMIC ACTION OF THE ANTIDIABETIC SULFANILAMIDES*

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It was shown in a previous communication, on the evidence of both in vitro and in vivo experiments, that the hypoglycemic effect of the antidiabetic preparation BZ-55 (nadizan, N-sulfanilyl-N^1-butyl-carbamide) was due to its inhibition of liver insulinase (an insulin-splitting enzymatic complex). The effect of this drug could not be demonstrated at all in depancreatized animals, while in alloxan-diabetic animals it was only effective if some residual insulin synthesizing capacity was retained in the pancreas. It was also found that, both in normal and in depancreatized animals, nadizan enhanced the hypoglycemic effect of introduced extraneous insulin. It was thus concluded that the hypoglycemic effect of the various sulfanilamide preparations could only take place when insulin, whether endogenous or exogenous, was actually present within the body.

However, a number of problems still remained unsolved: 1) did nadizan, as claimed by some authors [1, 5], exert any stimulating effect on the β-cells of the islet tissue of the pancreas; and, 2) was the enhancement by nadizan of the hypoglycemic action of insulin due entirely to its inhibitory effect upon insulinase, and consequently to the preservation of small amounts of the hormone from destruction, or was there also a specific peripheral activation of insulin by the drug.

The problem was thought capable of solution only under conditions where the islet tissue function was preserved completely, while liver insulinase was ineffective. If under such conditions nadizan should fail to exert its hypoglycemic effect, or to activate introduced extraneous insulin, it could be taken as evidence that the effect of the drug involved neither the islet tissue, nor the peripheral action of insulin itself, and that its hypoglycemic effect was due purely to insulinase inhibition. The present work was based on this theory.

EXPERIMENTAL METHODS

Liver damage was caused in experimental rabbits through subcutaneous injection of CCl_4 (1 ml/kg body weight, 3-5 injections at 48-hour intervals). Gross fatty infiltration was taken as a criterium of liver damage (increase in fat content, on dry weight, to 27-37%; normal value – 16.6 ±1.52%). The glycemic reaction to insulin (0.1 unit/kg), nadizan (0.3 kg) and the two factors given simultaneously was determined in all animals before and after poisoning. Liver insulinase activity was determined in both normal and poisoned animals by the method of Mirsky [3], and of Sklovskaia and Vol'kenzon [2], as follows. The livers were excised and placed into a Petri dish cooled in ice, and ice-cold 1% sodium chloride solution was perfused through a canula placed into the hepatic artery until the perfusate emerged clear. Portions of the washed liver, weighing 20-22 g, were chopped up and ground with an equal volume of cold physiological saline solution in the presence of quartz sand. The homogenate was pressed through cloth, the exudate was centrifuged and the filtered supernatant was dialyzed for 18 hours against distilled water. At the end of the dialysis period the precipitate was centrifuged off and the supernatant filtered. The filtrate thus obtained contained the albumin fraction of

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TABLE 1

The Effect of CCl_4-induced Liver Damage on Insulinase Activity and the Glycemic Reaction to Insulin

Rabbit No.	CCl_4 dose (in ml/Kg) X number of doses	Liver insulase activity (% decrease of initial glycemic)		Glycemic reaction to the introduction of 0.1 unit of insulin/ kg before CCl_4-poisoning				Glycemic reaction to the introduction of 0.1 unit of insulin/ kg after CCl_4-poisoning				liver fat, g%
		untreated extract	inactivated extract	initial blood sugar level, mg%	blood sugar level, Mg% at 1.5 hours	blood sugar level, Mg% 3 hrs.	maximal fall, in % of initial level	initial blood sugar level, mg%	blood sugar level, Mg% at 1.5 hours	blood sugar level, Mg% 3 hrs.	maximal fall, in % of initial level	
1	1×5	32.0	38.5	—	—	—	—	—	—	—	—	—
2	1×3	21.7	38.4	—	—	—	—	—	—	—	—	—
3	1×4	26.0	32.4	—	—	—	—	—	—	—	—	—
4	1×3	28.1	42.2	122	108	83	31.9	117	77	66	43.6	27.7
5	1×4	33.0	40.9	125	93	90	28.0	108	65	56	48.2	34.2
6	1×5	27.7	29.2	127	91	99	28.3	129	75	65	49.5	37.5
7	1×3	29.0	28.3	108	84	83	23.1	108	65	56	48.2	33.5
8	1×3	—	—	110	84	84	23.6	134	66	79	50.7	—
9	1×3	31.1	32.4	112	108	83	23.7	125	86	72	42.4	27.5
10	1×4	31.1	30.5	140	122	98	30.0	99	75	50	49.4	28.0
11	1×3	36.4	41.2	141	113	97	31.2	136	82	60	56.6	32.5
12	1×3	33.3	46.1	108	87	80	25.9	110	79	54	50.9	30.5
Mean		30.0	36.4	121	99	86	27.3	118	83	62	48,7	31.4

liver proteins, but not the low-molecular weight compounds like glutathione and cysteine, which are also capable of inactivating insulin; the extract was divided into two portions. Crystalline insulin, in amounts 1.5 units per 1 ml, was added to the first portion, the pH of the mixture was adjusted to 6.8 by the addition of the appropriate amount of Na_2HPO_4, and the whole incubated for one hour at 38°. The second portion was similarly treated, with the exception that the extract was inactivated by heating on the water bath for 30 minutes at 65-70°, before the addition of insulin. Insulin inactivation (and consequently, insulinase activity), was determined from the residual effect of the incubation mixture on the blood sugar of experimental rabbits. The incubation mixtures were given to rabbits subcutaneously, the dose being equivalent to 0.8 units of insulin initially present per kg. Blood samples were taken immediately before introduction of the reaction mixture and thereafter 1.5 and 3 hours after injection. Nadizan for intravenous injection was prepared as a 2.5% solution of the pure substance in an alkaline solution (pH 8) of sodium bicarbonate.

EXPERIMENTAL RESULTS

It was shown in the previous communication [1] that, while the introduction to rabbits of insulin previously incubated with inactivated dialyzed liver extracts caused a mean decrease in blood sugar of 30%, insulin incubated with intact liver extracts produced decrease in blood sugar of up to only 16% (mean value, 6%). It was thus possible to conclude, in agreement with Mirsky [3, 4], that normal rabbit liver contained an insulin-destroying enzymatic complex, which Mirsky termed insulinase.

It will be seen from the data presented in Table 1 that, in the majority of cases, the untreated extracts obtained from CCl_4-poisoned rabbits, when incubated with insulin and injected into test animals, produced an effect similar to that obtained with the inactivated normal liver extracts (the mean fall in blood sugar in the former case was 36.4%, in the latter — 30%). Thus, during fatty degeneration caused by CCl_4 poisoning, the liver was unable to destroy insulin, that is, the enzymatic complex insulinase was either absent from livers in

TABLE 2

The Effect of CCl_4-induced Liver Damage on the Hypoglycemic Activity of Insulin and Nadizan

Rabbit No.	Initial blood sugar level	Introduction of insulin	Blood sugar levels in mg%, before CCl_4 introduction, after an interval of				maximal decrease in % of initial levels	CCl_4 dose (in ml/kg) X number of doses	initial blood sugar level	Blood sugar levels in mg%, before CCl_4 introduction after an interval of				maximal decrease in % of initial levels
			15 min.	30 min.	1 hour	2 hours				15 min.	30 min.	1 hour	2 hours	
1	117	—0.1 units/kg . .	102	92	88	84	28.2	1×3	141	106	83	77	75	46.6
	119	—0.3/ kg	97	83	83	90	30.2	1×3	110	108	104	106	99	10.0
	127	—0.1 units/kg + —0.3/kg . . .	104	79	68	61	51.9	1×3	125	102	79	77	72	42.4
2	111	—0.1 units/kg . .	92	84	83	83	25.2	1×3	106	81	61	52	66	50.9
	120	—0.3/kg	93	79	68	63	47.5	1×3	113	113	115	108	104	7.9
	106	—0.1 units/kg + —0.3/kg . . .	70	65	57	48	54.6	1×3	124	97	86	72	63	49.2
3	101	—0.1 units/ kg . .	90	79	81	79	21.7	1×3	122	84	72	70	61	50.0
	124	—0.3/kg	93	79	68	70	45.1	1×3	115	113	117	110	104	9.5
	106	—0.1 units/ kg . .	70	65	57	48	54.6	1×3						
	115	—0.1 units/ kg + —0.3/ kg. .	93	77	65	54	53.0	1×3	104	79	68	57	66	54.7
6	119	—0.1 units/ kg . .	92	79	84	88	25.1	1×3	90	70	61	59	50	44.4
	107	—0.3/ kg.	80	69	63	60	49.3	1×3	83	83	75	74	81	10.8
	125	—0.1 units/kg + —0.3/ kg. . .	83	66	47	52	62.4	1×3	97	86	50	52	45	55.0
7	95	—0.1 units/ kg. . .	77	68	66	63	33.6	1×3	106	90	68	63	52	50.9
	86	—0.3/ kg.	65	54	57	53	37.2	1×3	105	100	100	93	93	11.4
	99	—0.1 units/ kg + —0.3/ kg . . .	75	50	56	48	51.5	1×3	104	79	68	59	56	46.2
8	103	—0.1 units/kg . .	86	78	77	81	21.3	1×3	103	90	63	47	62	54.3
	110	—0.3/ kg	102	97	87	74	32.7	1×3	119	112	112	111	114	6.7
	118	—0.1 units/ kg + —0.3/kg . .	99	83	75	68	42.4	1×3	115	76	61	53	61	53.9
4	102	—0.1 units/kg . .	81	77	79	77	24.5	1×3	97	81	68	47	50	51.4
	120	—0.3/ kg	83	81	68	66	45.0	1×3	113	110	111	106	102	9.7
5	95	—0.1 units /kg . .	74	73	74	70	26.3	1×3	88	74	57	47	7	47.1
	106	—0.3/ kg	84	75	61	65	42.4	1×3	92	92	90	84	88	13.5

this pathological state or, if present, had a very low activity.

It may be expected that the depression of insulinase activity, observed in the damaged liver, would result in a higher overall hypoglycemic effect of insulin, when given to poisoned animals. As seen from Table 1, this was actually the case; in all experiments, the single standard dose of insulin (0.1 unit/kg) was more effective in the animals suffering from carbon tetrachloride poisoning, than it was in the same animals before the administration of CCl_4 The mean fall in blood sugar caused by this dose in normal animals was 27.3%, while after CCl_4 poisoning the corresponding value rose to 48.7%.

In normal rabbits, prior to CCl_4 poisoning, nadizan given intravenously caused, within two hours, a fall in blood sugar of 30-45% (mean value, 40%); in CCl_4-poisoned animals the same dose of nadizan produced a fall in blood sugar of only 6.7-13.5% (mean value, 9.9%), that is, one within the limits of so-called spontaneous variations in blood sugar expected in any 2-hour period (Table 2).

Thus, during liver damage and the concomitant inactivation of insulinase, nadizan showed no hypoglycemic effect, even though the function and structure of the islet tissue remained intact; the reactivity to introduced insulin actually increased. In CCl_4-poisoned animals nadizan also failed to enhance the activity of added insulin, an effect invariably produced in these animals prior to poisoning.

Dulin and Johnston [6], using hepatectomized rats and dogs, maintained on infused glucose, observed a fall in blood sugar in these animals after introduction of tolbutamide (D-860) directly after operation. This effect was attributed to a stimulation of insulin secretion. It should, however, be borne in mind that, under such extremely un-physiological conditions, tolbutamide may have had a non-specific effect on blood sugar levels, particularly since hepatectomized animals develop hypoglycemia, at which stage glucose infusion stimulates insulin secretion. The present authors consider the experiments described above as providing a more satisfactory understanding of the role of liver in the hypoglycemic action of sulfonamides.

The present results thus established that under conditions of liver damage which lead to insulinase inactivation, nadizan had no hypoglycemic effect, nor did it enhance the effect of added insulin. The data show that nadizan has no effect on the peripheral action of insulin. The hypoglycemic effect of this drug is due to its inhibitory action on liver insulinase.

SUMMARY

Preparation BZ-55 caused hypoglycemia in normal rabbits, potentiated the hypoglycemic effect of insulin and inactivated insulinase of the liver. Insulinase is not found in rabbits with fatty dystrophy of the liver caused by carbon tetrachloride intoxication. The hypoglycemic effect of insulin is increased. Administration of BZ-55 (Nadizan) does not cause any hypoglycemic action and does not potentiate the effect of injected insulin, although the insular pancreatic apparatus is uninjured. These experiments permit to draw a conclusion that the hypoglycemic effect of BZ-55 is connected not only with the stimulation of the β-cells but also with the activation of the peripheral action of insulin. It is mainly caused by the inactivation of hepatic insulinase.

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