PHARMACOLOGY

THE IMPORTANCE OF THE INSULIN INACTIVATING PROPERTIES OF THE LIVER (INSULINASE) IN THE MECHANISM OF ACTION OF ANTIDIABETIC SULFONAMIDE PREPARATIONS

S. M. Leites and N. P. Smirnov

From the Division of Pathophysiology (Head – Prof. S. M. Leites) of the All-Union Institute of Experimental Endocrinology (Director – Prof. E. A. Vasyukova), Moscow

(Received September 30, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

We showed in a previous paper [1] that the hypoglycemic action of the sulfonamide antidiabetic preparation nadisan (BZ 55) is not shown in depancreatized dogs, and in dogs and rabbits with alloxan diabetes the action of the drug is only shown when a certain proportion of the β-cells are intact. In normal and depancreatized animals the administration of nadisan enhances the hypoglycemic effect of small doses of insulin. Thus the hypoglycemic action of nadisan may be revealed only in the presence of insulin in the body, either endogenously produced or administered from an external source. We also showed that the hypoglycemic effect of nadisan, and its activation of the action of insulin are accompanied by a fall in the activity of the insulinase of the liver. Our subsequent investigations [2] showed that in rabbits with toxic fatty dystrophy of the liver, caused by CCl_4 poisoning, the activity of the insulinase was considerably reduced, but the hypoglycemic effect of injection of small doses of insulin was increased. The hypoglycemia action of nadisan in rabbits with toxic fatty dystrophy of the liver is feebly expressed or absent. Nor does nadisan enhance the hypoglycemic action of injected insulin in these animals. Thus when the insulin apparatus is undamaged, the action of nadisan is greatly weakened if the activity of the insulinase in the liver is suppressed.

The feeble hypoglycemic activity of the sulfonamide preparations, or even its complete absence, in children suffering from diabetes is a well known fact. If, in fact, the hypoglycemic effect of nadisan is connected with its inactivation of the insulinase of the liver, it is natural to suggest that in young, uninjured animals the insulinase activity must be less pronounced than in adult animals, and the hypoglycemic action of nadisan weakened.

From this reasoning, in the present research we set out to investigate the insulinase activity of the liver at different age periods, determining at the same time the hypoglycemic effect of nadisan.

EXPERIMENTAL METHOD

Experiments were carried out on white rats of various ages: 1) before the onset of the period of sexual maturation (age not above 20 days, weight 30-50 g); 2) immediately after the onset of sexual maturation (weight 90-100 g); in adult rats (weight 170-250 g); 4) in old rats (weight 340-420 g). The insulin inactivating properties of the liver (insulinase) were determined, as in the previous investigations [1,2] by Mirsky's method. The insulinase activity was expressed by a value which was the inverse of the degree of lowering (in %) of the blood sugar in rabbits after injection of liver extract of rats, incubated with insulin, in relation to the control (liver extract, inactivated by heating, with the same dose of insulin).

EXPERIMENTAL RESULTS

As may be seen from Fig. 1, the insulinase activity of adult and old rats was reasonably well expressed (mean values 74.2 and 68.6), i.e., the degree of lowering of the blood sugar of the rabbit after injection of the liver extract of rats, incubated with insulin, was 74.2-68.6% weaker than in the control experiments in which the rabbit was injected with inactivated extract. In the sexually immature rats the power of the liver to inactivate insulin was much more weakly expressed, and in some animals it was even absent. On the average the insulinase activity was 15.

In rats reaching sexual maturity (weight 90-100 g), it was within the same limits as in the adult animals (on the average 68.8).

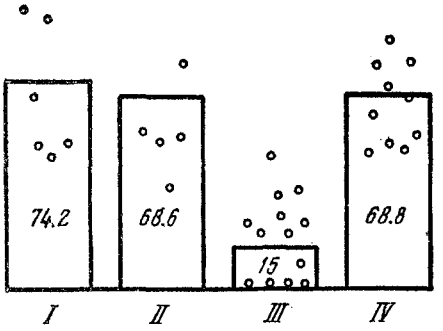

Fig. 1. Insulinase activity of the liver of rats of various ages. I) Adult rats weighing 180-230 g; II) old rats weighing 345-430 g; III) sexually immature rats weighing 30-40 g; IV) sexually mature rats weighing 90-110 g.

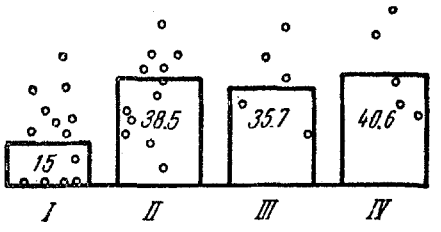

Fig. 2. The effect of injection of chorionic gonadotropin and sex hormones on the insulinase activity of the liver of sexually immature rats (weighing 30-40 g). I) Insulinase activity (control); II) the same, after preliminary injection of 25 mg of chorionic gonadotropin twice a day for 5 days; III) the same, after injection of testosterone propionate in a dose of 0.4 mg daily for 5 days; IV) the same, after injection of estradiol dipropionate in a dose of 100 i.u. daily for 5 days.

So far as the hypoglycemic action of nadisan is concerned, as may be seen from the results in Table 1, subcutaneous injection of 50 mg of this preparation per 100 g body weight into adult rats led to a clearly marked lowering of the blood sugar concentration 3 hours after injection: on the average from 103 to 63 mg%. In sexually immature rats, in which, as shown above, the insulinase activity was either feebly expressed or absent, injection of nadisan (60 mg/100 g body weight) had no hypoglycemic effect. In rats reaching sexual maturity, the hypoglycemic action of nadisan was revealed in the same way as in the adult rats (Table 1).

A definite parallel trend was thus observed in the sexually immature rats between the sharply reduced insulinase activity and the absence of hypoglycemic action of nadisan when the insulin apparatus was intact.

Since it was found in the experiments that in the sexually immature animals the insulinase activity and the hypoglycemic action of nadisan were greatly diminished or absent, it was necessary to establish to what extent this fact could be connected with the inadequate formation of sex hormones in these animals. In order to elucidate this problem we carried out the following experiments.

Sexually immature rats weighing 30-40 g were given injections of 25 mg of chorionic gonadotropin twice a day for 5 days, which led to a considerable increase in the reproductive system: the seminal vesicles and prostate gland in the males, growth of the uterus and ovaries (with hyperemia of the latter) in the females to a degree 6-7 times greater than in control animals. A second group of sexually immature male rats received subcutaneous injections of 0.4 mg of testosterone propionate daily for the same period of time, and a third group of female rats received 100 i. u. of estradiol dipropionate daily, also for a period of 5 days.

As may be seen from Fig. 2, injection of these hormones caused in all 3 groups of rats either intensification or appearance of insulin inactivating properties in the liver. The insulinase activity averaged 35.7-40.6, whereas in the control group (sexually immature rats not receiving injections of hormones) the insulinase activity was on the average 15. Injection of sex hormones to sexually immature rats thus enhanced their insulinase activity, although at the same time this activity did not

TABLE 1

The Hypoglycemic Action of Subcutaneous Injection of Nadisan to Rats of Various Ages

Age of rats	No. of animals in group	Wt of rat (in g)	Dose of nadisan (in mg/100 g body wt)	Initial blood sugar concentration (in mg %)	Blood sugar after 3 hr
Adult	8	180—230	50	103 (92—120)	63 (50—75)
Sexually immature	25	30—40	60	99 (79—122)	96 (74—117)
Sexually mature	8	90—100	50	108 (90—131)	65 (57—77)

Note (to Tables 1 and 2). Mean values are given. The limits of variation are shown in brackets.

TABLE 2

The Effect of Testosterone Propionate on the Hypoglycemic Action of Nadisan (50 mg/kg) in Adult Rats

Rats not receiving testosterone propionate				Rats receiving 2 mg testosterone propionate daily for 5 days			
No. of animals	wt. of rat (in mg%)	init. blood sugar conc. (in mg%)	blood sugar after 3 hours (in mg%)	No. of animals	wt. of rat (in mg%)	init. blood sugar conc. (in mg%)	blood sugar after 3 hours (in mg%)
8	180—230	103 (92—120)	63 (50—75)	8	170—250	103 (93—114)	60 (48—73)

Note. Insulinase activity of the first group of rats is 74.2, second is 66.8

reach its level in the sexually mature and adult animals. Parallel with the increased insulinase activity of the sexually immature rats subjected to the action of gonadotropin and sex hormones, the hypoglycemic action of nadisan began to be shown (Fig. 3).

The insulin inactivating power of the liver (the enzyme complex designated insulinase by Mirsky [5]) thus began to be revealed at a definite stage of postnatal development, and was formed in association with sexual maturity.

The injection of testosterone propionate to adult animals (2 mg/100 g body weight daily for 5 days) had essentially no effect on the insulinase activity of the liver nor on the hypoglycemic action of nadisan (Table 2). The sex hormones did not, therefore, directly activate insulinase, but merely promoted the formation of this enzyme system in the process of ontogenesis.

The results described in the present communication, like those of our previous investigation [2], show that the hypoglycemic effect of nadisan is connected with its inactivation of insulinase, and that this action does not appear when the insulin apparatus is intact, if the insulinase activity of the liver is not manifest or is weakened. Under these circumstances we do not rule out the possibility that some part may be played in the mechanism of the hypoglycemic action of sulfonamide preparations by their direct stimulating effect on the

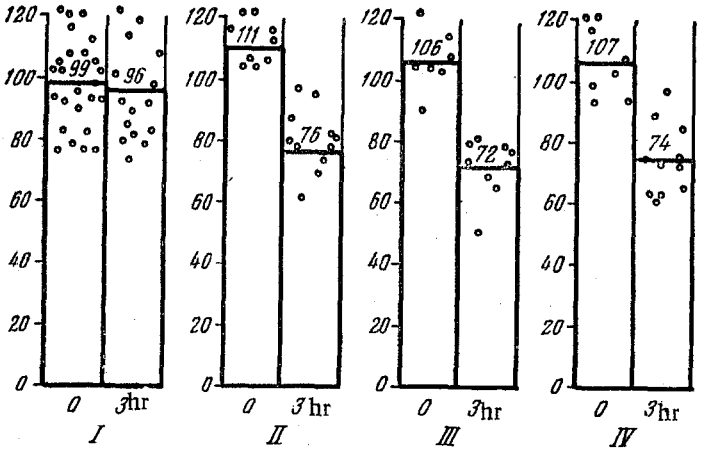

Fig. 3. The effect of chorionic gonadotropin and sex hormones on the hypoglycemic action of nadisan in sexually immature rats. I) Sexually immature rats (weight 30-40 g); II) the same after injection of 25 mg of gonadotropin twice a day for 5 days; III) the same after injection of testosterone propionate in a dose of 0.4 mg daily for 5 days; IV) the same after injection of estradiol dipropionate in a dose of 100 i. u. daily for 5 days.

β-cells of the islets of the pancreas, as indicated by the findings of Colwell and Colwell [3, 4]. This mechanism of the hypoglycemic action of the sulfonamides is not, however, the only one or the principal one, because, as our results from this present and from previous investigations showed, the hypoglycemic effect of nadisan is not shown even when it is still possible for it to stimulate the β-cells, if the insulinase activity of the liver is absent or considerably weakened. This gives grounds for the belief that the insulinase of the liver (and, possibly its other enzymes) is the fundamental component of the mechanism of the hypoglycemic action of the sulfonamides.

SUMMARY

Insulinase activity is either entirely absent or is considerably lower in sexually immature rats weighing from 30 to 50 gm. Nadisan (BZ 55) has no hypoglycemic effect in the animals of this age. It comes with the sexual maturity and then nadisan exerts a hypoglycemic effect in these animals. Administration of chorionic gonadotropine or sex hormones to sexually immature rats is associated with appearance or intensification of insulinase activity and hypoglycemic effect of nadisan. Injection of testosterone propionate to adult rats affects neither the hepatic insulinase activity nor the hypoglycemic effect of nadisan. This shows that sex hormones have no direct activating action on insulinase but only promote the development of this enzyme system during ontogenesis. These data may lead to the conclusion that inactivation of hepatic insulinase by sulfonamides plays an important role in the mechanism of their hypoglycemic effect.

LITERATURE CITED

[1] S. M. Leites and N. P. Smirnov, Probl. Endokrinol. i Gormonoterap., No. 4, 3-12 (1958).

[2] S. M. Leites and N. P. Smirnov, Byull. Eksptl. Biol. i Med., 46, No. 9, 53-56 (1958).*

[3] J. A. Colwell and A. R. Colwell, Diabetes, 1957, v. 6, N. 1, p. 60.

[4] J. A. Colwell and A. R. Colwell, Ann. of the New York Acad. Sc. 1957, v. 71, Art. I, p. 125.

[5] I. A. Mirsky et al., Metabolism, 1956, v. 5, p. 156-162.

*Original Russian pagination. See C.B. translation.