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Hypoglycemic Activity of Aldose Reductase Inhibitor, 1-[(*p*-Bromophenyl)sulfonyl]hydantoin

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The hypoglycemic activity of a potent aldose reductase inhibitor, 1-[(*p*-bromophenyl)-sulfonyl]hydantoin (*p*-Br–PSH), was tested. The compound was not effective when orally administered to rabbits at a dose of 25 mg/kg, whereas statistically significant hypoglycemic activity was observed at doses of 50 and 100 mg/kg. The hypoglycemic activity of *p*-Br–PSH, however, was lower than that of tolbutamide.

Keywords——aldose reductase inhibitor; hydantoin derivative; rabbit; hypoglycemic activity; diabetic complication; tolbutamide

Intracellular accumulation of D-glucitol, a reduction product formed from glucose by aldose reductase, has been implicated in the pathogenesis of diabetic complications such as cataract, peripheral neuropathy and vascular disease, particularly of the retina, kidney and heart.[1-3] Many aldose reductase inhibitors have been developed in an attempt to treat some of these complications.[4-7]

If an aldose reductase inhibitor has hypoglycemic activity, it should be a more promising drug for prevention and/or amelioration of diabetic complications. 1-[(*p*-Bromophenyl)-sulfonyl]hydantoin (*p*-Br–PSH) is one of the hydantoin derivatives which were shown to be potent aldose reductase inhibitors by the authors.[7] Since the structure of *p*-Br–PSH is reminiscent of those of hypoglycemic sulfonylureas, we decided to examine whether this compound has hypoglycemic activity. We report here that *p*-Br–PSH shows statistically significant hypoglycemic activity when orally administered to rabbits in doses of 50 and 100 mg/kg.

Materials and Methods

Administration of Drugs——Male rabbits (New Zealand albino, 2.0—3.0 kg), fasted for 12 h, were anesthetized by injecting sodium pentobarbital (Somnopentyl, Pitman-Moore) intraperitoneally at a dose of 26 mg/kg 1 h before administration of a test drug. Additional anesthetic was given at a dose of 13 mg/kg just after every awakening. *p*-Br–PSH (molecular weight, 319) was given to rabbits at doses of 25, 50, and 100 mg/kg and tolbutamide (molecular weight, 270) at a dose of 50 mg/kg. Hot water was gradually added to a mixture of *p*-Br-PSH and carboxymethyl cellulose sodium salt (2.5 : 1, 5 : 1, or 10 : 1, w/w) in a porcelain mortar with vigorous mixing to give a drug concentration of 7.5, 15, or 30 mg/ml for dosing at 25, 50, or 100 mg/kg, respectively. Sodium bicarbonate, an aid for facilitating drug absorption, was then added to the suspension to give a concentration of 6 mg/ml. A tolbutamide suspension containing sodium bicarbonate was similarly prepared. Drug suspensions were given by gastric intubation. An aqueous solution containing both sodium bicarbonate (6 mg/ml) and carboxymethyl cellulose sodium salt (3 mg/ml) was given to control animals.

Determination of Blood Glucose——Blood samples of each rabbit were obtained from the ear vein at specified

times during 5 h. Protein-free solutions were prepared from the blood samples according to the procedure of Somogyi.[8] Blood glucose was determined in duplicate with a kit, Blood Sugar-GOD-Perid-Test (Boehringer). Statistical significance was determined by means of Student's *t*-test, with P less than 0.05 as the criterion of significance.

Determination of Blood Insulin——Insulin in 0.1 ml of rabbit serum was assayed with a kit, Phadebas Insulin Test (Pharmacia Diagnostics) in which porcine insulin (as a standard) and anti-bovine insulin antibody raised in guinea pigs are used.

Purification and Measurement of Aldose Reductase——The purification of bovine lens aldose reductase and the measurement of the enzyme activity were performed as described previously.[7]

Results and Discussion

Figure 1 shows the effects of *p*-Br–PSH and tolbutamide (a control drug for comparison) on the blood glucose concentration of rabbits. Tolbutamide at 50 mg/kg markedly lowered the blood glucose level even at 1 h after administration. *p*-Br–BSH also showed statistically significant hypoglycemic activity when given at doses of 50 and 100 mg/kg. The hypoglycemic activity of *p*-Br–PSH, however, was lower than that of tolbutamide. Although *p*-Br–PSH lowered the blood glucose level even when given at a dose of 25 mg/kg, the effect was not statistically significant. Dose-response relationships in the hypoglycemic activity of *p*-Br–PSH were obtained at every time point from 2 to 5 h after administration.

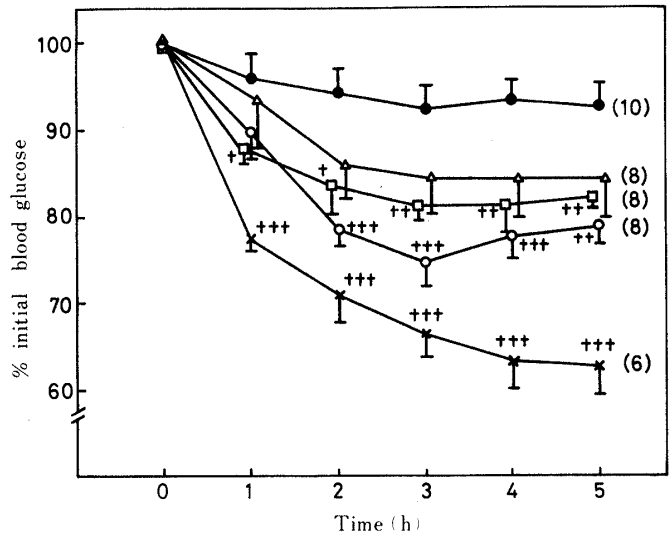

Fig. 1. Effects of *p*-Br–PSH and Tolbutamide on the Blood Glucose Concentration of Rabbits

p-Br–PSH was dosed *per os* at 25, 50, and 100 mg/kg, and tolbutamide at 50 mg/kg. The blood samples at zero time were taken just before the administration of the vehicle, *p*-Br–PSH or tolbutamide. The mean values of the glucose concentration in blood samples taken at zero time from control animals (●), *p*-Br–PSH-treated animals (△, 25 mg/kg; □, 50 mg/kg; ○, 100 mg/kg), and tolbutamide-treated animals (×) were 96.8, 98.3, 101.1, 98.7 and 97.9 mg/dl, respectively. The number of animals is shown in parentheses. Values and vertical bars represent means ± S.E.M. for the number of experiments noted. $^{+}p<0.05$ as compared to the control. $^{++}p<0.01$ as compared to the control. $^{+++}p<0.001$ as compared to the control.

The mechanism of action of sulfonylureas is still not clear, but it has been suggested that pancreatic insulin release is responsible for the hypoglycemic action of sulfonylureas.[9] We thus measured the blood insulin level of rabbits dosed with 100 mg/kg *p*-Br–PSH. *p*-Br–PSH, however, did not significantly increase the insulin level: 10.5 ± 1.7 μunits/ml (mean ± S.E.M., $n=6$) at zero time, 13.1 ± 1.6 μunits/ml ($n=6$) at the second hour, and 11.1 ± 1.8 μunits/ml ($n=6$) at the third hour. On the other hand, the insulin level was significantly increased (about

2.3 fold) by 50 mg/kg tolbutamide (a sulfonylurea drug) at 3 h after administration. These data suggest that the effect of *p*-Br–PSH is not necessarily mediated by a sulfonylurea-like action.

The basic active structure of hypoglycemic sulfonylureas can be depicted as A–SO_2–NH–B, with A being aryl and B being typically CONHR.[9] Holan and Samuel[10] attempted to build this structure into hydantoins and thus synthesized 3-alkyl-1-(arylsulfonyl)hydantoins. However, it has not yet been reported whether the compounds synthesized possess hypoglycemic activity.

Sorbinil (*S*-6-fluoro-spiro(chroman-4,4′-imidazolidine)-2″,5″-dione, a kind of spirohydantoin) is one of the most potent aldose reductase inhibitors so far known.[4] This compound at a dose of 20 mg/kg *per os*, however, was reported to have no effect on either fasting blood glucose or glucose tolerance in rats.[11] The values of inhibition percent of tolbutamide, chlorpropamide, and glibenclamide (10^{-5} M each) toward bovine lens aldose reductase were found to be 5, 8, and 23%, respectively. These drugs were markedly less potent than *p*-Br–PSH, which inhibited the enzyme by 50% at 3.7×10^{-7} M.[7]

Since the hypoglycemic activity of *p*-Br–PSH does not seem to be sufficiently high, we are continuing to search for much more potent compounds. The development of drugs having both inhibitory activity toward aldose reductase and hypoglycemic activity should be of great value in the treatment of diabetic complications.

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