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Inhibition of Sulphonylurea-Stimulated Insulin Secretion by Beta Adrenergic Blockade

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Summary. Normal dogs were injected i.v. with a single dose of 0.25 mg/kg sodium salt of HB 419 (Glibenclamide). Plasma insulin and glucose concentrations were measured at stated intervals over a period of two hours. The rise in insulin, but not the hypoglycemic response was abolished in peripheral blood when the animals were pretreated with a single i.v. injection of either 0.1 mg/kg or 0.3 mg/kg dl-propranolol, 30 min prior to the administration of HB 419. The d-isomer of propranolol was ineffective in this respect. These results indicate that a) the mechanism by which propranolol inhibits sulphonylurea-stimulated insulin secretion involves beta adrenergic receptors; and b) the hypoglycemia produced by HB 419 in the presence of propranolol could be the result of extrapancreatic effects. Since the possibility of an early rise in the insulin concentration of portal blood was not excluded in our present series of experiments, final proof will have to be provided by studies in totally pancreatectomized dogs.

Key words: Propranolol, HB 419, insulin, glucose, dogs.

Preliminary results from this laboratory have shown that a derivative of propranolol, Kö 592, was capable of inhibiting the insulinotrophic effect of HB 419 and tolbutamide in dogs [1, 2]. Since these blocking agents exert their metabolic effects occasionally by mechanisms other than by interaction with adrenergic receptors [3], the involvement of the adrenergic system, as suggested in our previous experiments, remained to be confirmed. Results of our present studies with racemic and d-propranolol in combination with HB 419 are providing further evidence in support of beta adrenergic control over sulphonylurea-stimulated insulin secretion. We found that the d-isomer, which interacts with the beta adrenergic receptor very poorly [3], was ineffective at dose levels at which the dl-preparation, containing the receptor-active l-isomer, inhibited sulphonylurea-stimulated insulin release.

Materials and Methods

Seven trained, conscious, metabolically normal mongrel dogs of either sex, weighing approximately 10 kg each, were used in these experiments. After an overnight fast, a single intravenous dose of 0.25 mg/kg sodium salt of HB 419 (Glibenclamide, Hoechst) was administered and plasma insulin concentrations were determined in peripheral venous blood at 15 min intervals for the first hour and at 30 min intervals for the second hour. Unpublished experiments conducted in our laboratory indicated that normal, trained, fasting, conscious dogs will respond to a single i.v. injection of a sulphonylurea by a gradual rise in peripheral plasma insulin concentration and that a lag period of about 15 min is required to encounter the first appreciable elevation. Insulin was measured by the double-antibody assay of Hales and Randle [4, 5], using the kit of the Amersham Searle Corporation; glucose concentrations were determined by the ferricyanide method of Hoffman [6], using the Technicon Autoanalyzer. Some of the animals also received racemic propranolol (Inderal, Ayerst), or the d-isomer (Ayerst) intravenously in doses of 0.1 mg/kg or 0.3 mg/kg body weight, 30 min prior to the administration of the sulphonylurea. Care was taken that the same animals receive once the dl- and then the l-compound with and without HB 419. Plasma insulin concentrations were expressed in absolute values as μU/ml of plasma and glucose concentrations were expressed as per cent change from absolute (mg/100 ml) values prevailing at zero time.

Results

Tables 1 and 2 give results of control experiments with dl- and d-propranolol. It will be noticed that propranolol alone in either form and dose level had no effect on plasma insulin concentrations. This is different from plasma glucose concentrations which showed a definite rise by nearly 30% in response to the two doses of dl-propranolol. No intrinsic hyperglycemic properties were encountered with the d-isomer:

Table 1. *Plasma insulin response to propranolol*

Mean ± S.E.M. at	0 time	15′	30′	45′	60′	90′	120′
dl-Propranolol 0.1 mg/kg i.v. n = 4	15 ± 1[a]	13 ± 1	14 ± 1	14 ± 1	14 ± 2	14 ± 1	15 ± 1
d-Propranolol 0.1 mg/kg i.v. n = 4	19 ± 4	14 ± 2	16 ± 1	18 ± 5	15 ± 2	12 ± 2	14 ± 1
dl-Propranolol 0.3 mg/kg i.v. n = 4	18 ± 1	15 ± 2	16 ± 2	17 ± 3	16 ± 3	16 ± 3	19 ± 4
d-Propranolol 0.3 mg/kg i.v. n = 4	15 ± 1	13 ± 0.4	13 ± 0.3	17 ± 2	15 ± 2	13 ± 0.3	14 ± 1

[a] Immunoreactive insulin μU/ml

Table 2. *Plasma glucose response to propranolol*

Mean ± S.E.M. at	0 time mg/100 ml	15′	30′	45′	60′	90′	120′
				Per cent differences			
dl-Propranolol 0.1 mg/kg i.v. n = 4	113 ± 4	+ 15 ± 3	+ 20 ± 5 $p < 0.025$[a]	+ 16 ± 6 $p < 0.10$	+ 17 ± 4 $p < 0.025$	+ 12 ± 7	+ 6 ± 3
d-Propranolol 0.1 mg/kg i.v. n = 4	114 ± 3	0 ± 1	+ 3 ± 1	+ 2 ± 0.2	+ 1 ± 1	− 2 ± 1	− 2 ± 1
dl-Propranolol 0.3 mg/kg i.v. n = 4	111 ± 3	+ 13 ± 5	+ 28 ± 5 $p < 0.005$	+ 30 ± 6 $p < 0.01$	+ 21 ± 7 $p < 0.05$	+ 25 ± 6	+ 19 ± 8
d-Propranolol 0.3 mg/kg i.v. n = 4	112 ± 3	+ 4 ± 2	+ 3 ± 2	+ 5 ± 1	+ 1 ± 2	− 2 ± 1	− 2 ± 1

[a] All p values are calculated by comparison with d-propranolol at corresponding dose and time.

fluctuations in plasma glucose concentrations were identical to those seen in saline-injected animals (omitted from table).

The results of combination experiments with propranolol and HB 419 are shown on Tables 3 and 4. It is evident that HB 419-stimulated insulin secretion was abolished by racemic propranolol even at the small dose of 0.1 mg/kg. This is in contrast with the d-isomer, the "receptor-inactive" preparation, which was not capable of modifying appreciably the insulinotrophic effect of the sulphonylurea. Regardless of the form of propranolol used for pretreatment, administration of HB 419 produced a reduction in plasma glucose concentration. The hypoglycemic response to dl-propranolol and HB 419 was less pronounced than that seen after HB 419 alone, but it is noteworthy that a reduction in plasma glucose concentration was encountered at all in the presence of virtually unchanged peripheral plasma insulin concentrations. Although it is not indicated in the table, the hypoglycemic response to dl-propranolol and HB 419 was statistically highly significant if compared either with saline-injected controls or with the hyperglycemic response to dl-propranolol alone.

Discussion

It is well recognized that beta adrenergic blockade can be effectively achieved with small doses of l-propranolol but not with the d-isomer [3]. In all probability in doses that are higher than those employed in our present experiments, the latter would also be effective in producing a blockade, but under these conditions mechanisms other than interaction with adrenergic receptors may become dominant [3]. Thus, the difference in response to HB 419 combined with relatively small doses of dl- and d-propranolol suggests very strongly that beta adrenergic receptors are involved in controlling the insulinogenic response to sulphonylureas.

The reduction in blood glucose concentration as a response to d-propranolol and HB 419 administration can be attributed to enhanced insulin secretion, but the reduction obtained after dl-propranolol and HB 419 may have occurred for other reasons because the peripheral plasma insulin concentration did not change. Potentiation of the action of insulin by sulphonylureas which has been described [7, 9] and the

Table 3. *Plasma insulin response to HB 419 with and without propranolol*

Mean ± S.E.M. at	0 time	15′	30′	45′	60′	90′	120′
HB 419 0.25 mg/kg i.v. n = 7	17 ± 1[a]	32 ± 3	32 ± 3	32 ± 4	25 ± 2	21 ± 2	20 ± 3
dl-Propranolol 0.1 mg/kg and HB 419 n = 4	15 ± 2[c]	21 ± 5	17 ± 2 $p < 0.01$[b]	18 ± 2 $p < 0.05$	20 ± 3	17 ± 3	18 ± 4
d-Propranolol 0.1 mg/kg and HB 419 n = 3	16 ± 3[c]	45 ± 9	35 ± 4 $p > 0.5$	44 ± 5 $p > 0.1$	37 ± 7	29 ± 3	23 ± 2
dl-Propranolol 0.3 mg/kg and HB 419 n = 4	15 ± 3[c]	19 ± 3	18 ± 4 $p < 0.025$	15 ± 2 $p < 0.025$	14 ± 2	16 ± 4	15 ± 3
d-Propranolol 0.3 mg/kg and HB 419 n = 4	14 ± 1[c]	26 ± 1	35 ± 8 $p > 0.5$	29 ± 3 $p > 0.5$	29 ± 2	38 ± 3	25 ± 5

[a] Immunoreactive insulin μU/ml.
[b] All p values are calculated by comparison with HB 419 alone at corresponding times.
[c] Zero time = injection of HB 419 which is 30 min after propranolol.

Table 4. *Plasma glucose response to HB 419 with and without propranolol*

Mean ± S.E.M. at	0 time mg/100 ml	15′	30′	45′	60′	90′	120′
			Per cent differences				
HB 419 0.25 mg/kg i.v. n = 7	114 ± 2	−24 ± 5	−46 ± 4	−51 ± 2	−51 ± 2	−44 ± 4	−38 ± 4
dl-Propranolol 0.1 mg/kg and HB 419 n = 4	138 ± 5[b]	−17 ± 3	−31 ± 3 $p < 0.05$[a]	−33 ± 4 $p < 0.001$	−38 ± 4 $p < 0.025$	−41 ± 2	−41 ± 3
d-Propranolol 0.1 mg/kg and HB 419 n = 3	116 ± 4[b]	−51 ± 4	−43 ± 8 $p > 0.5$	−50 ± 6 $p > 0.5$	−50 ± 5 $p > 0.5$	−50 ± 4	−43 ± 3
dl-Propranolol 0.3 mg/kg and HB 419 n = 4	132 ± 8[b]	−16 ± 6	−34 ± 8 $p < 0.2$	−32 ± 4 $p < 0.001$	−31 ± 4 $p < 0.005$	−29 ± 4	−26 ± 7
d-Propranolol 0.3 mg/kg and HB 419 n = 4	115 ± 2[b]	−14 ± 4	−28 ± 7 $p < 0.05$	−39 ± 7 $p > 0.05$	−44 ± 5 $p > 0.2$	−46 ± 5	−45 ± 4

[a] All p values are calculated by comparison with HB 419 alone at corresponding times.
[b] Zero time = injection of HB 419 which is 30 min after propranolol.

concomittant reduction in hepatic glycogenolysis due to beta adrenergic blockade [1] could explain the fall in the concentration of plasma glucose. On the other hand, it was mentioned before that the same beta blocker alone caused under our experimental conditions, nearly a 30% increase in the blood sugar level, i.e. an effect more in line with stimulated hepatic glucose output than with its inhibition. The hypoglycemic action of sulphonylureas in the presence of normal peripheral insulin values and its relationship to beta adrenergic blockade require clarification on a number of points. For instance, the possibility of an early rise in insulin concentration of the portal blood was not excluded in our studies and experiments in totally pancreatectomized dogs are now in progress.

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