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Effects of Propanolol and Sotalol on Epinephrine-Induced Hyperglycemia and Glycogen Depletion of Liver and Muscle in the Rat (38322)

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Two seemingly opposed kinds of observations have been made on the effects of β-adrenergic antagonists on the actions of epinephrine on carbohydrate metabolism. The first series of observations reported that the hyperglycemic response to epinephrine in the rat was significantly reduced by several β-adrenergic antagonists, including dichloroisoproterenol [DCI] (Claaser and Noach (1); Northrop and Parks (2)), propranolol [Inderal] (Kvam *et al.* (3)), and sotalol [MJ-1999] (Kvam *et al.* (3); Kennedy and Ellis (4); Stanton (5)). The second observation is that one β-adrenergic antagonist, dichloroisoproterenol, in large doses did not modify epinephrine-induced liver glycogenolysis in the rat (Kennedy and Ellis (6); Ellis (7)).

The observation that dichloroisoproterenol did not inhibit epinephrine-induced liver glycogenolysis, but did reduce epinephrine-induced hyperglycemia could be suggested as a special case since the other β-adrenergic antagonists have not been tested for their ability to inhibit epinephrine-induced liver glycogenolysis. Another possibility is that in the rat the plasma glucose change is a poor indicator of the change in liver glycogenolysis. The latter would be the case if the β-adrenergic antagonists do not influence the liver glycogenolysis but do reduce the plasma glucose and the muscle glycogenolysis in response to epinephrine.

In this paper we attempt to clarify the actions of the β-adrenergic antagonists in the rat by determining the effects of two potent β-adrenergic antagonists, propranolol and sotalol administered in large doses, on epinephrine-induced hyperglycemia and glycogen depletion in the liver and in skeletal muscle of fasted rats.

Methods. White female rats weighing between 175 and 225 g were fasted for approximately 24 hr prior to use. The animals were anesthetized with pentobarbital sodium, 35 mg/kg, and subsequent small doses of pentobarbital were administered as required to maintain anesthesia. All drug administrations were by the intraperitoneal (ip) route. Animals were divided into four groups: a control group, which received saline (0.9% NaCl) instead of a drug at the designated intervals; the epinephrine-treated group, which received a saline injection at the appropriate time prior to the epinephrine administration; the antagonist-treated group, which was given a saline injection instead of epinephrine at the designated period after the antagonist; and the antagonist agonist-treated group which received both a β-adrenergic blocking agent and an epinephrine injection. All were similarly treated. The β-adrenergic antagonist, propranolol or satolol, or saline was injected at approximately 30 min after the administration of the pentobarbital. Thirty min after the injection of the antagonist or saline epinephrine bitartrate or saline was administered. Blood and tissue samples were obtained one hour after the administration of epinephrine or saline.

Blood was obtained by cutting the tip of the tail just once prior to the first sample. Blood samples were obtained approximately 5 min before the administration of each test substance or saline and just prior to obtaining the tissue samples. In order to avoid the difficulties in obtaining blood from the tail after the administration of epinephrine and the differences between cardiac and tail blood when hyperglycemia and vascular constriction occur in response to a drug, terminal blood samples were obtained by cardiac puncture. An 0.1 ml sample of blood was diluted 1:10 and analyzed for glucose by the method of Hoffman (8) as modified for the Technicon Autoanalyzer.

Drug solutions in the concentrations indicated were prepared in 0.9% saline at the beginning of

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each experiment: propranolol hydrochloride, 10 mg/ml; sotalol hydrochloride, 20 mg/ml; and epinephrine bitartrate, 1 mg/ml. Suitable dilutions in 0.9% saline were made, when necessary, just prior to the injection. All drug doses are expressed in mg/kg in terms of the weight of the respective salt of the drug.

Results. The data summarized in Fig. 1 shows that 90 min after the administration of propranolol at doses of 5 and 30 mg/kg (ip) blood glucose was not elevated above that of the control group level. Epinephrine bitartrate, 0.2 mg/kg, at 1 hr after its administration produced an increase in the blood glucose of about 80 mg/100 ml above that in the control group. Epinephrine in the same dose administered to animals pretreated with propranolol, 5 mg/kg or 30 mg/kg, produced elevation of blood glucose levels of approximately 30 and 40 mg/100 ml, respectively. Since 30 mg/kg appeared to be the largest dose of propranolol which most rats could tolerate under the stated conditions, propranolol *in vivo* is evidently able to antagonize only a portion of the hyperglycemia induced by epinephrine.

Effect of propranolol on epinephrine-induced muscle glycogenolysis. Muscle glycogen content did not appear to be affected by propranolol, 5–30 mg/kg (Fig. 1). Epinephrine (Fig. 1) reduced the glycogen content of rat gastrocnemius muscle about 35% from that found in the control group. The muscle glycogen contents of the groups pretreated with propranolol 30 min prior to the administration of epinephrine were at about the same level as that found for the saline-treated controls. Thus, propranolol at these doses appeared to inhibit completely the epinephrine-induced depletion of muscle glycogen.

Effect of propranolol on epinephrine-induced liver glycogenolysis. Propranolol in the large doses administered did not appear to modify significantly the glycogen content of the rat liver (Fig. 1). Epinephrine depleted approximately 85% of the liver glycogen level observed in the control, or saline-treated group. Pretreatment of the animals with propranolol, 5–30 mg/kg, prior to the administration of epinephrine did not show any inhibition of the epinephrine-induced depletion of liver glycogen level when the propranolol plus epinephrine-treated group is compared with the epinephrine-treated group. Thus, epinephrine-induced glycogenolysis in the liver was not inhibited by propranolol.

Effects of sotalol on epinephrine-induced hyperglycemia. Kvam *et al.* (3) reported that sotalol (MJ-1999) at a dose of 10 mg/kg antagonized partially epinephrine-induced hyperglycemia in the rat. In the present experiments, a ten-fold larger dose of sotalol, 100 mg/kg, had no effect itself on the blood glucose level and even this dose only reduced the epinephrine-induced hyperglycemia about 60% (Fig. 2). The data reported by Kvam *et al.* (3) for a lower dose of sotalol and the present study with a much larger dose indicate that this β-adrenergic antagonist also produces a far from complete antagonism to epinephrine-induced hyperglycemia

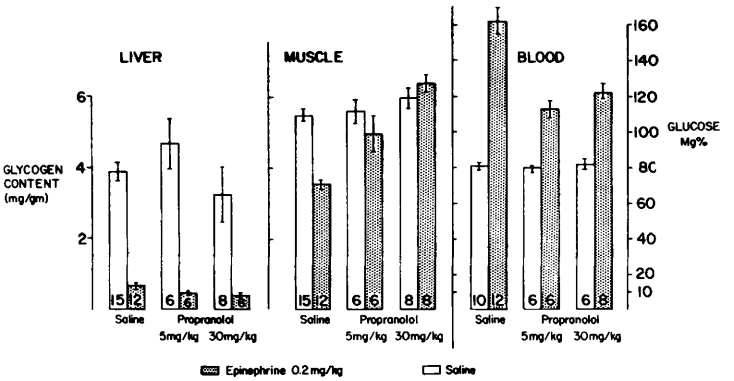

FIG. 1. Effects of large doses of propranolol on the reductions in liver and muscle glycogen and on the increase in blood glucose produced by epinephrine in the rat. Each column topped by a bar represents the mean value and the standard error of the mean (± SE) for each group. The numeral at the bottom of each column indicates the number of rats included in the group. The rats were anesthetized with pentobarbital. Either propranolol or saline was administered ip 30 min before the ip injection of epinephrine or saline and samples of tissue or blood were obtained 60 min after the second injection.

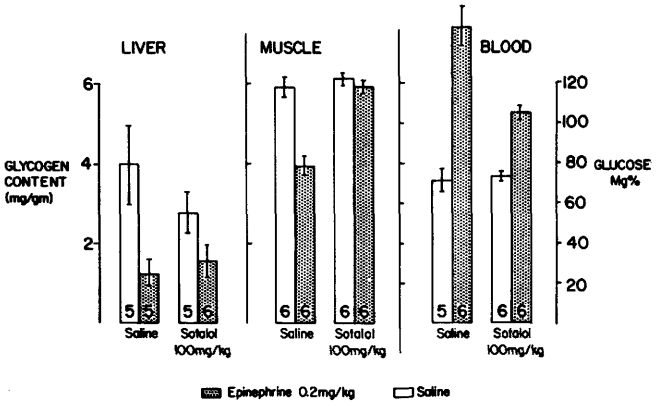

FIG. 2. Effect of a large dose of sotalol on the reductions in liver and muscle glycogen and on the increase in blood glucose produced by epinephrine in the rat. See Fig. 1 for details.

in the rat.

Effect of sotalol on epinephrine-induced muscle and liver glycogenolysis. Figure 2 shows that sotalol, 100 mg/kg, did not itself modify the level of muscle glucogen, but this large dose of sotalol did completely antagonize epinephrine-induced glycogenolysis in the muscle.

Sotalol, 100 mg/kg, alone did produce a decrease in the liver glycogen, but the change was not statistically significant. Even at this high dose (Fig. 2) sotalol did not prevent epinephrine-induced liver glycogenolysis.

Discussion. In these studies in fasted rats the administration of epinephrine bitartrate, 0.2 mg/kg ip, approximately doubled the blood glucose level in 60 min. Similar results in the rat have been reported previously (2, 9, 10). Pretreatment with propranolol, 5 mg/kg or 30 mg/kg, did not completely antagonize the epinephrine-induced hyperglycemia. It is noteworthy that the rats which received the largest tolerated dose, 30 mg/kg, still exhibited a blood glocuse rise which was approximately 40% above the control level. Stanton (5) has reported that a much lower dose of propranolol, less than 0.1 mg/kg ip, in the rat reduced epinephrine-induced hyperglycemia about 30%. The much higher doses of propranolol used in these studies (Fig. 1), 50- and 300-fold greater than the dose used by Stanton, produced a maximum antagonism of the hyperglycemia of about 60%.

The epinephrine-induced hyperglycemia in rats in these studies was accompanied by muscle and also liver glycogenolysis. The β-adrenergic antagonists, propranolol (Fig. 1) and sotalol (Fig. 2), reduced the hyperglycemic response to epinephrine and prevented the glycogen depletion of muscles, but did not inhibit the epinephrine-induced glycogenolysis in the rat liver. These data demonstrate that two potent β-adrenergic antagonists, propranolol and sotalol, can partially antagonize epinephrine-induced hyperglycemia in the rat without concomitantly reducing the hepatic glycogenolytic effect of epinephrine. The results thus extend the previous findings with the first β-adrenergic antagonist, dichloroisoproterenol with which Claassen and Noach (1) had observed a partial antagonism of epinephrine-induced hyperglycemia in the rat and Kennedy and Ellis (6) had found no antagonism of epinephrine-induced hepatic glycogen depletion.

Since the β-adrenergic antagonists decreased, but did not completely inhibit the hyperglycemic response and did not reduce the hepatic glycogenolytic response to epinephrine, the mechanism of the reduction in the hyperglycemic response may be related to the inhibition of the muscle glycogenolysis and the reduction in plasma substrates for gluconeogenesis. Epinephrine-induced muscle glycogenolysis was prevented in these studies by the β-adrenergic antagonists propranolol (Fig. 1) and sotalol (Fig. 2), as in previous studies DCI (6, 11, 12, 13), pronethalol (14, 15), IMA (15) and propranolol (16) had inhibited catecholamine-induced muscle glycogenolysis. These findings with β-adrenergic antagonists, the observation that the α-adrenergic antagonists do not inhibit catecholamine-induced muscle glycogenolysis (6) and the much greater potency

of isoproterenol than epinephrine in activating muscle glycogenolysis (17) and phosphorylase (14) indicate that the muscle adrenergic receptors satisfy Ahlquist's original criteria (18) for a β-adrenergic receptor.

Despite the clear evidence that β-adrenergic antagonists exhibited no antagonism to epinephrine-induced hepatic glycogenolysis in the intact rat (Figs. 1 and 2; also (6)) and that the α-adrenergic antagonists were not effective for inhibiting catecholamine-induced hepatic glycogenolysis, there are data obtained from other experimental approaches which support the proposal that this hepatic receptor is a subtype of the β-adrenergic receptor (7). Thus, the β-adrenergic antagonists DCI (11), methoxamine (11) and pronethalol (14) antagonized the activation of rat liver phosphorylase activity by epinephrine and, in other studies, sotalol (3) partially inhibited the increase in cyclic AMP. Also, propranolol in quite high concentrations competitively antagonized epinephrine-induced glucose release from rat liver slices (Ellis, unpublished observations). Recently, Newton and Hornbrook (19) presented strong evidence for a β-adrenergic receptor associated with adenyl cyclase in the rat liver. Thus, the evidence indicates that catecholamines-induced hepatic glycogenolysis in the rat involves a β-adrenergic receptor, but *in vivo* the receptor appears quite insensitive to blockade by the presently available potent β-adrenergic antagonists.

The present observations with propranolol and sotalol on epinephrine-induced liver and muscle glycogenolysis appear consistent with the earlier observation in the rat with the β-adrenergic antagonist DCI reported by Kennedy and Ellis (6). The data presented show that propranolol or sotalol only partially antagonize the epinephrine-induced hyperglycemic response in the rat without a concomitant antagonism of the epinephrine-induced hepatic glycogen depletion. The partial inhibition of the epinephrine-induced hyperglycemia must therefore be related to the inhibition of muscle glycogenolysis since there was no indication that propranolol, even at just sublethal doses, could reduce the action of epinephrine on the rat liver.

Summary. Propranolol at doses of 5 and 30 mg/kg and sotalol in a dose of 100 mg/kg only partially inhibited the hyperglycemic response to epinephrine in the rat. These β-adrenergic antagonists completely antagonized the glycogenolytic effect of epinephrine on skeletal muscle, but they did not modify the marked effect of epinephrine on liver glycogen.

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