Inhibitory Effect of Propranolol on Hyperglycemia Induced by Dibutyryl 3′,5′-Cyclic AMP (36190)

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(Introduced by M. A. Greer)

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Exton *et al.* (1) have suggested that glucose output from the liver may be regulated by agents which increase tissue levels of 3′,5′-cyclic AMP and by insulin, which lowers the level of this nucleotide. Since that time, the glycogenolytic action of 3′,5′-cyclic AMP has been well documented (2–7). In view of the facts that the hyperglycemic action of epinephrine can be blocked by β-adrenergic blocking agents (8–10), and that epinephrine may manifest its hyperglycemic action by increasing the level of 3′,5′-cyclic AMP (2), it is possible that the hyperglycemic action of 3′,5′-cyclic AMP may be blocked by β-adrenergic blocking agents. In the present study, dibutyryl 3′,5′-cyclic AMP and propranolol were the agents chosen for testing the above hypothesis.

Materials and Methods. One hundred and twenty-four male rats, weighing approximately 200 g, were used in this experiment. The animals were fed laboratory chow and water *ad lib.* Beginning 8–20 hr before the experiment, the diet was withdrawn. Graded doses of dibutyryl 3′,5′-cyclic AMP (DBcAMP) dissolved in 1 ml of 0.9% saline were injected intravenously and 1 ml of blood was obtained by cardiac puncture just before, 30, 60 and 120 min after injection of DBcAMP. When propranolol (Ppl) was used, 0.1 mg of Ppl in 1 ml of saline was injected intravenously 20–90 min before injection of DBcAMP. Blood glucose concentration was measured with an autoanalyzer.

Results. Effect of DBcAMP on blood glucose. The effect of graded doses of DBcAMP on blood glucose was measured in three experiments. Fasting blood glucose concentration did not vary greatly from experiment to experiment and from group to group (Table I). In saline injected controls, a slight but progressive increase of blood glucose was found. When 2 or 4 mg of DBcAMP were administered, no significant increase of blood glucose was found 30–60 min after administration. However, 8–10 mg of DBcAMP caused a significant hyperglycemia. In agreement with the previous report (5), the doses of DBcAMP used were devoid of any visible untoward effect on the animals.

Effect of propranolol on DBcAMP-induced hyperglycemia. In experiment 1, Ppl (0.1 mg) or saline was injected intravenously 90 min before obtaining the first blood sample. Soon after obtaining the first blood sample, saline or DBcAMP was injected intravenously. Blood samples were then obtained 30, 60 and 120 min after DBcAMP administration. As shown in Exp. 1A of Table II, blood glucose increased 60–120 min after administration of 8 mg of DBcAMP, the increase being comparable to those found in Exp. 1D and Exp. 2E of Table I. This increase of blood glucose was slightly less 60 min after DBcAMP in Exp. 1B in which 0.1 mg of Ppl was administered 90 min before injection of DBcAMP. However, the difference was of questionable significance ($.05 > p > .1$).

In experiment 2, Ppl (0.1 mg) or saline was injected 20 min before DBcAMP administration. Administration of DBcAMP again produced an increase of blood glucose 30–60 min later (Exp. 2A of Table II). This increase of blood glucose was prevented by previous administration of Ppl (Exp. 2B of Table II).

In order to study a possible hypoglycemic action of Ppl, serial blood samples were ob-

TABLE I. Effect of DBcAMP on Blood Glucose.[a]

Group	Injected materials	No. of rats	Blood glucose (% of initial blood sample) before injection	30 min after	60 min after	120 min after
Exp. 1 A	Saline	8	100 (116.9 ± 3.4)		110.0 ± 7.8	131.0 ± 10.2
B	DBcAMP (2 mg)	7	100 (103.1 ± 4.2)		120.6 ± 12.8	146.6 ± 10.9
C	DBcAMP (4 mg)	6	100 (96.9 ± 3.5)		124.0 ± 14.9	130.3 ± 9.4
D	DBcAMP (8 mg)	6	100 (102.5 ± 3.8)		165.2 ± 14.3	147.8 ± 17.5
Exp. 2 E	DBcAMP (8 mg)	13	100 (108.7 ± 3.4)		168.2 ± 7.0	153.8 ± 11.1
F	DBcAMP (8 mg)	11	100 (104.6 ± 4.3)	158.8 ± 7.2 (158.2 ± 15.6)	224.9 ± 11.1	
Exp. 3 G	Saline	10		(112.2 ± 3.7)		
H	DBcAMP	10		(250.1 ± 11.4)		

[a] Values are expressed as mean ± SE of mean. Parentheses indicate actual value of blood glucose. DBcAMP = dibutyryl 3′,5′-cyclic AMP was injected intravenously soon after obtaining the initial blood sample. DBcAMP used in Exp. 1 and 2 was obtained from Kyowa-Hakko Ltd, and that used in Exp. 3 was obtained from Boehringer Japan Ltd. The diet was omitted beginning 8 hr (Exp. 1 and 3) or 12 hr (Exp. 2) before experiment.

Statistical analysis: Exp 1 A–D 60 min after $.01>p>.001$
Exp 2 F 30 min–60 min $.001>p$
Exp 3 G–3H 30 min after $.001>p$

tained after administration of 0.1 mg Ppl. Ppl produced slight hypoglycemia (80% of control) but this effect was short-lasting and almost disappeared by the time of DBcAMP administration.

Discussion. Conversion of inactive phosphorylase to active phosphorylase in the liver leads to breakdown of glycogen to glucose-1-phosphate with hepatic mobilization of glucose (11). The increase in active phosphorylase by the action of epinephrine in liver slices may be produced by stimulation of adenyl cyclase and consequent elevation of 3′,5′-cyclic AMP. Although the glycogenolytic action of 3′,5′-cyclic AMP is well established, it is found that DBcAMP is more potent than cyclic AMP possibly because of good penetration of DBcAMP through the cell membrane (2). A number of studies have indicated that β-adrenergic blocking agents, particularly Ppl, can block the hyperglycemic action of epinephrine (8–10), possibly by preventing a stimulatory action on the adenyl cyclase–cyclic AMP system. Similarly, Ppl blocks the stimulatory effect of TSH (12) and vasopressin (13) which are mediated through a stimulation of adenyl cyclase. In view of these findings, it can be expected that Ppl will prevent hyperglycemia produced by the administration of cyclic AMP. In contrast to this hypothesis, Levine and Vogel (4) have reported that pretreatment with rather small doses of Ppl (0.1–0.5 mg/kg) failed to prevent hyperglycemia produced by cyclic AMP. Therefore, we set out to study in groups of rats how β-adrenergic blocking agent, Ppl, would modify hyperglycemia produced by DBcAMP. In contrast to the previous studies (14, 15), in which a long lasting hypoglycemia was produced by continuous infusion of Ppl, only a temporary hypoglycemia was produced by a single injection in our experiments. The effect almost disappeared 20 min after injection of Ppl. However, even after the direct hypoglycemic action of Ppl had disappeared, Ppl blocked the hyperglycemia produced by DBcAMP. Since no untoward effect of Ppl was found in our experiment, and since other

TABLE II. Effect of Propranolol on DBcAMP-Induced Hyperglycemia.[a]

Group	Injected materials	No. of animals	Blood glucose (% of initial blood glucose) before injection	30 min after	60 min after	120 min after
Exp. 1 A	Saline + DBcAMP (8 mg)	8	100		170.9 ± 4.2	159.8 ± 13.0
B	Ppl + DBcAMP (8 mg)	8	100		147.4 ± 10.0[b]	163.9 ± 8.8
Exp. 2 A	Saline + DBcAMP (8 mg)	11	100	158.8 ± 7.2	224.9 ± 11.1	
B	Ppl + DBcAMP (8 mg)	10	100	107.7 ± 5.6	119.1 ± 6.0[c]	

[a] Values are expressed as mean ± SE of mean. DBcAMP (dibutyryl 3′,5′-cyclic AMP) was injected intravenously soon after obtaining the first blood sample. DBcAMP used in Exp. 1 was obtained from Kyowa-Hakko Ltd, and that used in Exp. 2 was obtained from Boehringer Japan Ltd. Ppl = propranolol (0.1 mg) was injected 90 min (Exp. 1) or 20 min (Exp. 2) before DBcAMP administration. The diet was omitted beginning 20 hr before the experiment.

Statistical analysis:

[b] $.05 < p < .1$.

[c] $p < .001$ when compared to its respective control.

studies clearly indicated a specific inhibitory action of Ppl (2, 12), it seems unlikely that our present finding is due to a nonspecific toxic effect.

Summary. DBcAMP significantly increased blood glucose within 30 min in the rat without showing any side effects. This increase of blood glucose was inhibited when 0.1 mg of Ppl was injected 20 min before administration of DBcAMP. When a similar dose of Ppl was injected 90 min before administration of DBcAMP, Ppl failed to inhibit the increase of blood glucose produced by DBcAMP.

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