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DISTURBANCE OF INSULIN RELEASE IN BORDETELLA PERTUSSIS VACCINATED RATS

By

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It is well known that treatment with *Bordetella pertussis* vaccine (BPV) increases the sensitivity of rats to histamine, 5-hydroxytryptamine (5-HT) and anaphylactic shock (10, 15). The development and the severity of allergic and anaphylactic responses are considerably influenced by alteration in the carbohydrate metabolism (1, 3, 17). In rats the administration of BPV results in a decrease of blood sugar (3, 6, 12) and in an inhibition of epinephrine induced hyperglycaemia (6, 12) both of which are probably connected with the development of a hypersensitive state. The diminished hyperglycaemic response may be explained, at least in part, by the presence of hyperinsulinism (6), and by the fact that epinephrine increases the insulin levels in BPV-treated, fasted rats as opposed to its normal action in untreated controls (6, 19).

In the present work the effect of BPV on the insulin response to various agents has been studied and an elevated activity of insulin secretion mechanism was found following BPV-treatment.

METHODS

Wistar female rats (150-200 g) received standard diet and drinking water *ad libitum* and in all cases were fasted for 18 hours prior to plasma insulin examinations. BPV was administered i.p. in a single dose of 3×10^{10} organisms 4 days before insulin determinations. Blood samples were taken from the right carotic artery before and 30 min. after administration of catecholamines—epine-

phrine, norepinephrine and isoproterenol (each in a dose of 2 mg/kg s.c.)—20 min. after theophylline (75 mg/kg i.p.) and 15 min. after the injection of the α -blocking agent, phentolamine (50 mg/kg i.p.). Glucose (150 mg/kg) was administered intravenously and plasma insulin was determined at various intervals as indicated in Table 2.

All experiments were performed under pentobarbital anaesthesia. Plasma insulin levels were measured by the radioimmunoassay of *Hales & Randle* (7) and the amount was expressed as μ U/ml. The results were statistically evaluated by Student's *t* test.

RESULTS

In BPV-treated rats the basal insulin levels were somewhat higher than in normal controls (Table 1 and 2). Nevertheless, in our experiments this increase was not so consequent as observed by *Gulbenkian et al.* (6).

TABLE 1

Insulin Response to Catecholamines, Phentolamine and Theophylline in Normal and BPV-treated Fasted Rats.

Treatment	Control		BPV-treated	
	Insulin μ U/ml $\pm$ SE	No. of rats	Insulin μ U/ml $\pm$ SE	No. of rats
Epinephrine				
before	46 $\pm$ 5		61 $\pm$ 7	
after	33 $\pm$ 5 $\S$	9	403 $\pm$ 32*	6
Norepinephrine				
before	40 $\pm$ 4		55 $\pm$ 6	
after	29 $\pm$ 3 $\S$	8	181 $\pm$ 25 $\dagger$	6
Isoproterenol				
before	29 $\pm$ 3		51 $\pm$ 5	
after	157 $\pm$ 13*	6	1290 $\pm$ 50*	6
Phentolamine				
before	22 $\pm$ 4		65 $\pm$ 13	
after	77 $\pm$ 13 $\S$	7	824 $\pm$ 78*	7
Theophylline				
before	41 $\pm$ 5		90 $\pm$ 13	
after	212 $\pm$ 16*	11	890 $\pm$ 21*	8

* $p < 0.001$, $\S$ $p < 0.01$, $\dagger$ $p < 0.02$, $\$$ $p < 0.05$
(one tailed *t* test) compared to the values obtained before drug administration.

In intact animals plasma insulin was decreased by epinephrine or norepinephrine (Table 1). After BPV pretreatment the effect of epinephrine and norepinephrine was reversed, i.e., a marked elevation of insulin levels could be observed following the administration of these catecholamines. The other examined drugs—the predominantly β -agonist isoproterenol, the α -blocking agent phentolamine and theophylline—all raised the plasma insulin concentration both in control and BPV-treated groups. However, the elevation was far higher in BPV pretreated rats.

Physiological saline alone did not cause any significant changes either in control or in BPV-treated animals. These results are not shown in the tables. Following intravenous administration of glucose, a sharp rise ensues in the plasma insulin levels with a maximum after 1.5 min. (Table 2). The insulin response to glucose seems to be somewhat higher in the BPV-treated group, however, the differences between BPV-treated and control animals were by no means so pronounced when using drugs indicated in Table 1.

TABLE 2

Insulin Response to Glucose in Normal and BPV-treated Fasted Rats.

	Plasma insulin $\mu\text{U/ml} \pm \text{SE}$	
	Control (6)	BPV-treated (5)
Before glucose administration	46 $\pm$ 4	83 $\pm$ 8
Minutes after glucose administration		
1.5	74 $\pm$ 6§	163 $\pm$ 8*
5.0	52 $\pm$ 2	115 $\pm$ 11
10.0	54 $\pm$ 5	120 $\pm$ 14
15.0	55 $\pm$ 5	114 $\pm$ 14

* $p < 0.001$, § $p < 0.01$

(one tailed t test) compared to values obtained before glucose administration. Numbers in parenthesis represent the number of animals.

DISCUSSION

It is generally accepted that the regulatory effect of catecholamines on insulin secretion is mediated by a fall or an increase

in the cyclic AMP level of the islets as a result of α - or β -adrenergic stimulation (2, 20). Both epinephrine and norepinephrine are known to inhibit insulin secretion by stimulating α -adrenergic receptors (2, 8, 9, 13, 22). This normal action of the above-mentioned two catecholamines was reversed after BPV sensitisation, i.e., in BPV treated rats they significantly increased plasma insulin levels. As it was previously shown in *in vitro* experiments isoproterenol, a predominantly β -agonist stimulated insulin secretion of rat pancreatic tissue (5, 8). The *in vivo* observations of *Tabachnick & Gulbenkian* (19) as well as our present findings revealed that isoproterenol increases insulin secretion both in normal and in BPV-treated rats, but the elevation was far higher in the latter case. Recently *Senft et al.* reported that if the α -adrenergic stimulatory effect of endogenous catecholamines was blocked by the α -blocking agent phentolamine, the remaining β -adrenergic stimulatory effect of endogenous catecholamines leads to an increase in insulin secretion (16). The insulin response to endogenous catecholamines, similarly to exogenous catecholamines, was also increased after BPV-treatment as it was verified by our phentolamine experiments.

These results are inconsistent with the theory of *Fishel & Szentiványi* (4) which suggests that BPV sensitization elicits the blockade of β -adrenergic receptors. On basis of our present findings two possible explanations could be given for the disturbed insulin release of BPV-treated rats. First, a blockade of α -adrenergic receptors develops following the administration of BPV, consequently the prevailing β -antagonist action of catecholamines stimulates insulin secretion. Secondly, BPV causes an elevated activity or sensitivity of one or more steps in the β -receptor mediated insulin secretion pathway. Thus, in BPV-treated animals the stimulatory action of catecholamines highly surpass their inhibitory effect. The latter hypothesis seems to be confirmed by our results regarding theophylline. It has been reported earlier that theophylline, acting as a phosphodiesterase inhibitor, increased cyclic AMP level in the pancreatic islets (20), and stimulated insulin secretion in rats

(21). As shown in Table 1 the insulin response to theophylline was also very much increased by BPV pretreatment. Similar observations were made by *Tabachnick & Gulbenkian* in fed rats (19). These facts suggest that BPV influences the insulin secretion mechanism behind the receptor level. Naturally, further experiments will have to be carried out to clarify this problem in detail.

At the current stage of our knowledge we are aware of two biochemical pathways of insulin secretion: a) the chain of glucose and glycolysis, b) the chain of the β -adrenergic receptors, of adenyl cyclase and of cyclic AMP (8, 14). Naturally, there exist correlations between the two chains. Insulin secretion regulated essentially by glucose is subject to the modulatory action of the adrenergic mechanism. We demonstrated that insulin response to glucose and basal insulin levels were only moderately affected by BPV. Therefore it could be assumed that BPV acts primarily on the cyclic AMP mediated chain, and the altered modulatory effect of endogenous catecholamines could result in the observed increase of basal insulin levels, and of glucose-stimulated insulin release.

The increase of insulin secretion would appear to play a role in the inhibition of epinephrine (6, 12) and isoproterenol (11) induced hyperglycaemia observed in BPV-treated rats. Therefore it may be one of the factors increasing sensitivity to anaphylaxis and shock mediators in pertussis sensitized rats. The demonstrated disturbance in insulin secretion could exert its inhibitory effect on catecholamine induced hyperglycaemia in two ways: partly, by increasing peripheral glucose utilization, and partly by acting on liver glycogen metabolism (18).

In our opinion, subsequent thorough investigations on altered insulin release of BPV-treated rats could lead to a possible explanation of the inhibition of catecholamine induced hyperglycaemia, and could also be a useful tool for clarifying some details of the insulin secretion mechanism.

SUMMARY

The effect of BPV pretreatment on plasma insulin response to various agents was investigated in rats. The normal inhibitory action of epinephrine and norepinephrine was reversed in BPV-treated animals. BPV highly increased the insulin response to isoproterenol, phentolamine and theophylline. Glucose induced insulin release and basal insulin levels were only moderately elevated by BPV. The disturbed insulin secretion and its influence on carbohydrate metabolism may play an important role in the pathomechanism of increased anaphylactic sensitivity following BPV administration.

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