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THE EFFECT OF BORDETELLA PERTUSSIS VACCINE ON PROTEASE SENSITIVITY AND ON THE ANAPHYLACTIC PROTEASE PRODUCTION IN RATS

By

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Adrenalectomy and Bordetella pertussis vaccine (BPV) are commonly used to decrease the resistance of rats to anaphylactic shock (3, 6, 8, 12). While the preliminary injection of BPV enhances the susceptibility to active but not to passive anaphylaxis, adrenalectomy increase the sensitivity to both types of anaphylactic shock (9).

The pathomechanism of rat anaphylaxis and of the increased sensitivity produced by BPV is not well known, but protease systems are known to play an important role in various inflammatory and allergic reactions (14). Our previous study showed that adrenalectomy increased anaphylactic protease release parallel with the development of severe shock symptoms, and besides, that adrenalectomy enhanced the sensitivity to proteases (10). These findings suggested that proteases may be important factors in rat anaphylaxis and in the increased anaphylactic sensitivity caused by adrenalectomy.

Assuming certain similarities between the pathomechanism behind the effect of adrenalectomy and BPV treatment, the present experiments have been carried out to determine the effect of BPV on protease sensitivity, and on the anaphylactic protease release.

MATERIALS AND METHODS

Wistar albino rats (200–250 g) were fed with a standard diet. Drinking water was allowed ad libitum. BPV was administered intraperitoneally in a single dose of 3×10^{10} organisms.

Protease shock was produced by i.v. injection of various doses of twice crystallized bovine pancreas trypsin (REANAL, Budapest) or *Streptomyces* pronase P (SERVA, Heidelberg) dissolved in 0.5 ml of physiological saline. BPV pretreated rats were injected with protease 5 days after pertussis inoculation. The values of LD_{50} were determined using 50 animals in each group examined.

Heterologous passive and active anaphylaxis were carried out as previously described (10). The antigen (4-times crystallized ovalbumine) and the purified rabbit antiovalbumin were prepared in our laboratory. Some groups of passively sensitized animals were given BPV 5 or 12 days before being challenged. Since no difference could be detected between the two groups, only the results of animals injected 5 days before shock challenge were presented in the figures. Two groups of actively sensitized rats received BPV together with the first sensitizing antigen dose. In preliminary experiments we determined the severity of anaphylactic shock in each of the anaphylactized groups.

Animals subjected to anaphylactic shock were killed 10 minutes after being challenged and measurements of acid and neutral proteolytic serum activity were immediately carried out. To determine whether BPV alone could influence serum protease levels, rats killed 5 (BPV₅) or 12 (BPV₁₂) days after a single BPV injection were also examined. Serum acid and neutral protease levels were determined by a modified ninhydrin-hydrindantin procedure as previously described (10). The results were expressed as μmol of amino groups released by 0.1 ml serum per hour, which was considered to represent 1 unit of enzyme activity. The values in the figures show the average of 8 samples and the standard deviations. Statistical analysis was made by Student's *t* test.

RESULTS

There were no significant differences in the sensitivity to trypsin or pronase between control and BPV treated groups (Table 1).

In the absence of BPV, only mild shock symptoms developed in rats. According to the findings of *Mota* (9), BPV treatment failed to modify the severity of heterologous passive anaphylactic shock. However, as it was detected in preliminary experiments, active anaphylactic shock in rats injected with BPV at the time of sensitization was so severe that 90 per

TABLE 1.

Effect of BPV Treatment on the Sensitivity of Rats to Trypsin and Pronase.

Animals	LD ₅₀ (mg/kg)*	
	Trypsin	Pronase
BPV treated	24	26
Control	22	32.5

* Death in 4 hours was taken into account.

cent of the animals died within 4 hours with typical haemorrhagic lesions in the intestines.

BPV alone did not cause any changes in serum acid protease levels (Fig. 1). In agreement with our previous results (10) both passive and active anaphylaxis significantly increased the acid protease activity in the serum. Anaphylactic acid protease release was not influenced by previous BPV treatment.

Neutral proteolytic activity changed only in the serum of animals undergoing active anaphylactic shock (Fig. 2). There was only a minor elevation in neutral protease level during active anaphylaxis. On the other hand, a significant rise of neutral protease activity could be observed in serum of BPV pretreated and actively anaphylactized animals.

DISCUSSION

Acid protease measured by haemoglobin splitting method is considered to be the lysosomal endopeptidase cathepsin D (7). The elevated serum level of this enzyme indicates lysosomal damage. The participation of released lysosomal hydrolases and other substances in the pathomechanism of inflammatory and hypersensitive reactions is generally accepted (4). In mouse anaphylaxis a reduction in hepatic lysosomal enzymes and an elevation of plasma acid phosphatase has been observed by *Treadwell* (13). Furthermore, in mice an anaphylactoid state can be induced by the intravenous injection of

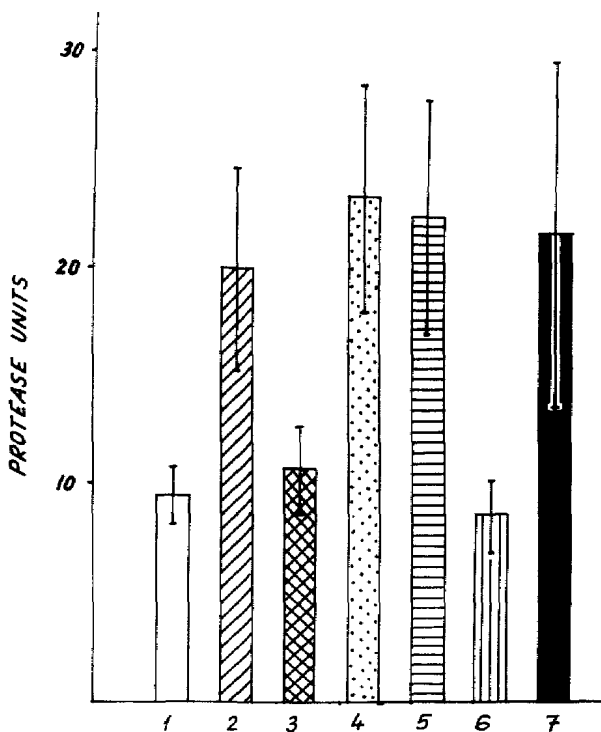


Fig. 1.

The effect of active and passive anaphylaxis on serum acid protease levels in normal and BPV treated rats. 1) Control, 2) Active anaphylaxis, 3) BPV₁₂, 4) BPV + active anaphylaxis, 5) Passive anaphylaxis, 6) BPV₆, 7) BPV + passive anaphylaxis.

Values of significance:

$$p_{1,2} < 0.001, p_{1,4} < 0.001, p_{3,4} < 0.001, p_{1,5} < 0.001,$$

$$p_{1,7} < 0.001, p_{6,7} < 0.001.$$

hepatic lysosomal enzymes (13). Rats also have been shown to undergo significant alterations in hepatic acid phosphatase during anaphylaxis (5). We were also able to detect an increase of lysosomal acid protease activity in serum during

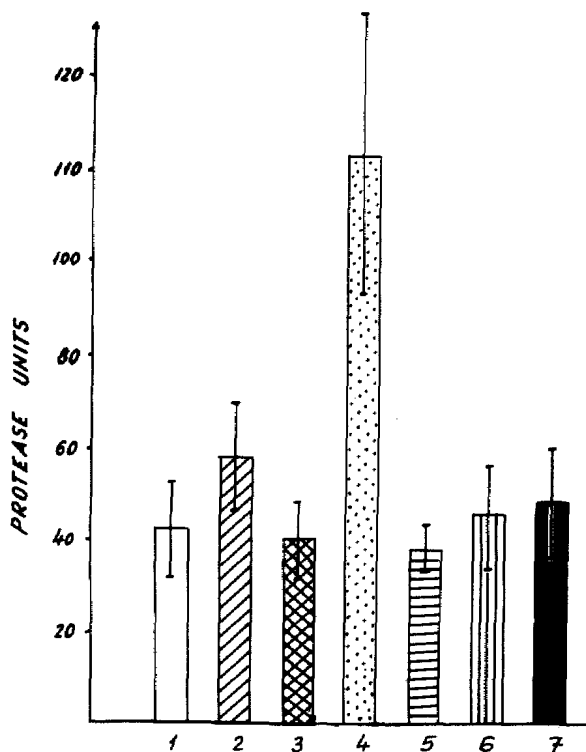


Fig. 2.

The effect of active and passive anaphylaxis on serum neutral protease levels in normal and BPV treated rats. 1) Control, 2) Active anaphylaxis, 3) BPV_n, 4) BPV + active anaphylaxis, 5) Passive anaphylaxis, 6) BPV_s, 7) BPV + passive anaphylaxis.

Values of significance:

$$P_{1,2} < 0.05, P_{1,4} < 0.001, P_{3,4} < 0.001.$$

anaphylaxis. The observed lysosomal damage may be one of the factors responsible for the development of mild shock symptoms in rats. However, BPV pretreatment did not increase anaphylactic acid protease release, even in actively

sensitized animals, in spite of the fact that these animals produced dramatic shock symptoms following challenge. These results suggest that lysosomes did not play an important role in the pathomechanism of severe active anaphylaxis.

In our previous report it was shown that serum neutral protease activity increased in severe active and passive anaphylaxis of adrenalectomized rats (10). In addition, the present experiments have demonstrated an elevation of serum neutral protease levels in BPV treated and actively anaphylactized rats. These findings suggest a close relationship between the development of severe anaphylactic shock and the elevated neutral protease levels. On the basis of these findings we conclude that neutral proteases, which are able to elicit tissue injury by the formation of plasma kinins, or by acting directly on structural tissue components (14), may play an important part in severe anaphylactic symptoms and in the mechanisms of increased sensitivity caused by adrenalectomy or BPV treatment. The nature and origin of neutral proteases released or activated during anaphylaxis is not known as yet. Further experiments will have to be carried out to clarify this problem.

Our earlier investigations regarding the histamine, 5-hydroxytryptamine, and carbohydrate metabolism (1, 2, 11) demonstrated some similarities between the effect of adrenalectomy and BPV treatment. It was supposed that reversible adrenal insufficiency developed after pertussis sensitization would be responsible—at least in part—for the effects of BPV. However, the examination of anaphylactic protease production showed that BPV pretreatment and adrenalectomy caused similar changes only in serum neutral protease activity of actively anaphylactized animals. The lack of parallels in the other examined cases may be explained by the quantitative and qualitative differences between total adrenalectomy and reversible adrenocortical insufficiency, or by possible additional effects of BPV which may overlap the consequences of adrenal hypofunction.

SUMMARY

The sensitivity of rats to exogenous proteases (trypsin, pronase) was not influenced by BPV treatment. Acid protease activity of serum was increased during active and heterologous passive anaphylactic shock. BPV pretreatment did not alter serum acid protease levels either in normal or in anaphylactized rats. The neutral protease levels were markedly elevated only in BPV pretreated rats undergoing severe, active anaphylactic shock. The role of proteases in rat anaphylaxis is discussed.

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