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HYPOTENSION IN RABBITS INFECTED WITH *Trypanosoma brucei*

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1 Blood pressures and heart rates of 12 anaesthetized rabbits chronically infected with *T. brucei* were measured (average infection time 39 days (range 25–67)). The systolic BP was 31.4 ± 5.7 mmHg, the diastolic BP 25.0 ± 7.2 mmHg, and the heart rate 120.5 ± 24.2 beats/minute. Two rabbits were already hypotensive 10 days after infection. In 12 anaesthetized control rabbits, the systolic BP was 66.2 ± 7.3 mmHg (mean ± s.e.), the diastolic BP 60.2 ± 7.3 mmHg, and the heart rate 116.3 ± 15.9 beats/minute.

2 The intravenous injection of 3×10^8 disintegrated trypanosomes into infected rabbits lowered the blood pressure by 41.4 ± 22.0%. Pretreatment of two rabbits with aprotinin prior to administration of parasites prevented the fall in blood pressure.

3 Injection of 3×10^8 live trypanosomes complexed with hyperimmune sera produced a fall of 68.3 ± 38.4% in the systolic BP of normal rabbits. Disintegrated or live trypanosomes, or hyperimmune sera alone had no effect. Pretreatment of animals with aprotinin prior to administration of the immune complex abolished the fall in BP.

4 The results suggest that the profound hypotension in chronic trypanosomiasis is caused by complex formation of trypanosomes with antibody. Since it can be prevented by pretreatment with aprotinin, it is likely that activation of plasma kallikrein with a subsequent release of plasma kinins contributes to this effect.

Introduction

Chronic infections of *Trypanosoma brucei* in the rabbit are characterized by the appearance of a succession of antigenic variants each of which stimulates the formation of its own antibody (Gray, 1962). Each rise in antibody titre corresponds to the disappearance of the homologous antigen variant. It is probable that, at each peak of parasitaemia, a reaction between the trypanosome and its variant antibody occurs which results in the removal of most of the trypanosomes from the circulation. This trypanosome/antibody complex formation has been implicated in the release of plasma kinins known to occur in trypanosomiasis (Boreham, 1968a, 1970). *In vitro* studies have shown that when trypanosomes are mixed with immune sera the resulting complexes are able to liberate kinins from a suitable substrate (Boreham & Goodwin, 1970). The mechanism of release appears to be the activation of Hageman factor by the immune complex which in turn converts prekallikrein to kallikrein, the kinin-forming enzyme (Boreham, 1968b; Boreham & Goodwin, 1970). It has been suggested that the release of kinins *in vivo* may have important pathological effects such as increasing vascular permeability (Goodwin, 1970) but to date proof of any such effect is lacking. In an attempt to provide such evidence, the effect of trypanosome complexes on blood pressure and the influence of a kallikrein inhibitor on this effect have been investigated.

Methods

Rabbits were lightly anaesthetized with urethane (1.2 g/kg intravenously) and the blood pressure measured from the left carotid artery with a Bell and Howell electronic transducer. Test substances were administered through a cannula in the right jugular vein in 1 ml volumes and washed in with 0.2–0.4 ml sterile 0.9% w/v NaCl solution (saline). All animals were treated with heparin (1000 units/kg).

Trypanosoma brucei strain 427 were separated from heavily infected rat blood on DEAE cellulose

columns by the method of Lanham (1968). Complexes of trypanosomes with antibody were prepared by incubating 3×10^8 separated trypanosomes with 2 ml of rabbit serum hyperimmune against *T. brucei* at 37°C for 30 min with gentle agitation. The suspension was centrifuged at 800 *g* for 5 min and the sediment washed twice in sterile phosphate buffer pH 7.4, before resuspending in buffer. Trypanosomes were disintegrated by repeated freezing and thawing.

Results

Blood pressure and heart rates were measured in 12 control New Zealand white rabbits. The systolic pressure was 66.2 ± 7.3 mmHg, the diastolic pressure 60.2 ± 7.3 mmHg and the heart rate 116.3 ± 15.9 beats/minute. In 12 rabbits, chronic infection with *T. brucei* 427 (average infection time 39 days, range 25–67) was accompanied by profound hypotension. The systolic pressure was 31.4 ± 5.7 mmHg, the diastolic pressure 25.0 ± 7.2 mmHg and the heart rate 120.5 ± 24.2 beats/minute.

In two rabbits which had been infected for only ten days the blood pressures were 46/40 and 36/30 mmHg with heart rates of 136 and 124 beats/min respectively.

Intravenous injections of separated whole or disintegrated trypanosomes, or of trypanosomes heated at 96°C for 30 min had no significant effect on the blood pressure of normal rabbits even when the number injected was as high as 10^9. Occasionally, a rapid transient fall of blood pressure lasting not more than 5 min and not exceeding about 15% was seen which was attributed to vascular reflexes caused by blockage of small vessels. However, when 3×10^8 disintegrated trypanosomes were injected intravenously into chronically infected rabbits a significant ($41.4 \pm 22.0\%$), often biphasic fall in blood pressure occurred 3–5 min later in all ten animals. It persisted for at least 10 min (Figure 1a). In most cases the blood pressure remained low for 30 min, when the experiment was terminated.

In two chronically infected rabbits (day 31) pretreated with aprotinin 5000 iu/kg 5 min before administration of 3×10^8 disintegrated parasites, the mean fall in blood pressure was only 5.2%.

The effect of trypanosome/antibody complexes on 6 non-infected control rabbits produced a fall of $68.3 \pm 38.4\%$ in blood pressure (Figure 1b). The fall started 3–5 min after injection of the complexes. Three of these rabbits died within 15 min of administration of the complexes. Pretreatment of three non-infected rabbits with aprotinin completely abolished the hypotensive effect of complexes. Antiserum or live trypanosomes alone had no effect on blood pressure of non-infected rabbits.

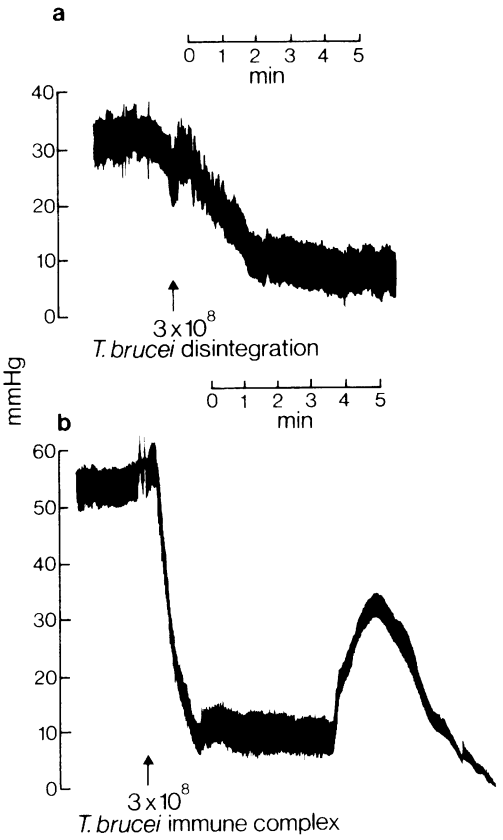

Figure 1 (a) Effect of intravenous injection of 3×10^8 disintegrated *T. brucei* on blood pressure of an anaesthetized rabbit chronically infected with *T. brucei.* (b) Effect of intravenous injection of 3×10^8 *T. brucei* + antibody immune complex suspension on blood pressure of an anaesthetized normal rabbit.

Discussion

Rabbits infected with trypanosomes developed severe hypotension as early as ten days after infection but showed no change in heart rate. Injections of trypanosomal antigen into chronically infected rabbits, or of immune complexes of trypanosomes and antibody into control rabbits, caused falls in blood pressure beginning 3–5 min after the injections. The falls in blood pressure were attributed to immune complexes because trypanosomes or immune sera alone had no effect on the blood pressure of non-infected rabbits. It is known that high levels of both variant and common antibodies are present as early as one week after infection (Gray, 1962). In these experiments the antigen used for intravenous injection was the same as that used to infect the rabbits, thus

antibody would be present at the time of the experiment. The resulting immune complexes may produce the release of hypotensive substances from the host. The findings support earlier *in vitro* work (Boreham & Goodwin, 1970).

The fall in blood pressure in both experimental situations was inhibited by aprotinin which suggests that the hypotension was mediated by kallikrein. Aprotinin inhibits activated kallikrein but does not prevent the activation of prekallikrein or Hageman factor (Vogel & Werle, 1970). These results are consistent with the view (Boreham & Goodwin, 1970) that particulate complexes of trypanosomes with antibody adsorbed on to their surface activate Hageman factor which converts prekallikrein to kallikrein; the latter then forms kinins.

Various particles with negative charges, e.g. glass, kaolin and extracellular membranes, activate Hageman factor bound to the negatively charged surface, by a process of limited proteolysis (Wuepper & Cochrane, 1972; Cochrane, Revak & Wuepper, 1973); a variety of particulate and non-particulate immune complexes did not activate the kinin system in rabbit or human plasma, although some bacterial contaminants were able to do so (Cochrane, Wuepper, Aikino, Revak & Spiegelberg, 1972). Our results do not agree with these findings since trypanosomes alone did not cause hypotension or release kinin *in vitro* (Boreham & Goodwin, 1970).

Both kallikrein (Depot Glumorin, 4 k.u.) and bradykinin (0.2 μg) produce a fall in blood pressure of about 30% in rabbits. Thus the hypotension seen in trypanosome infections of the rabbit could be caused either by kallikrein or kinin. The present results are the first *in vivo* evidence suggesting that the release of pharmacologically active substances contributes to the pathology of trypanosomiasis.

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