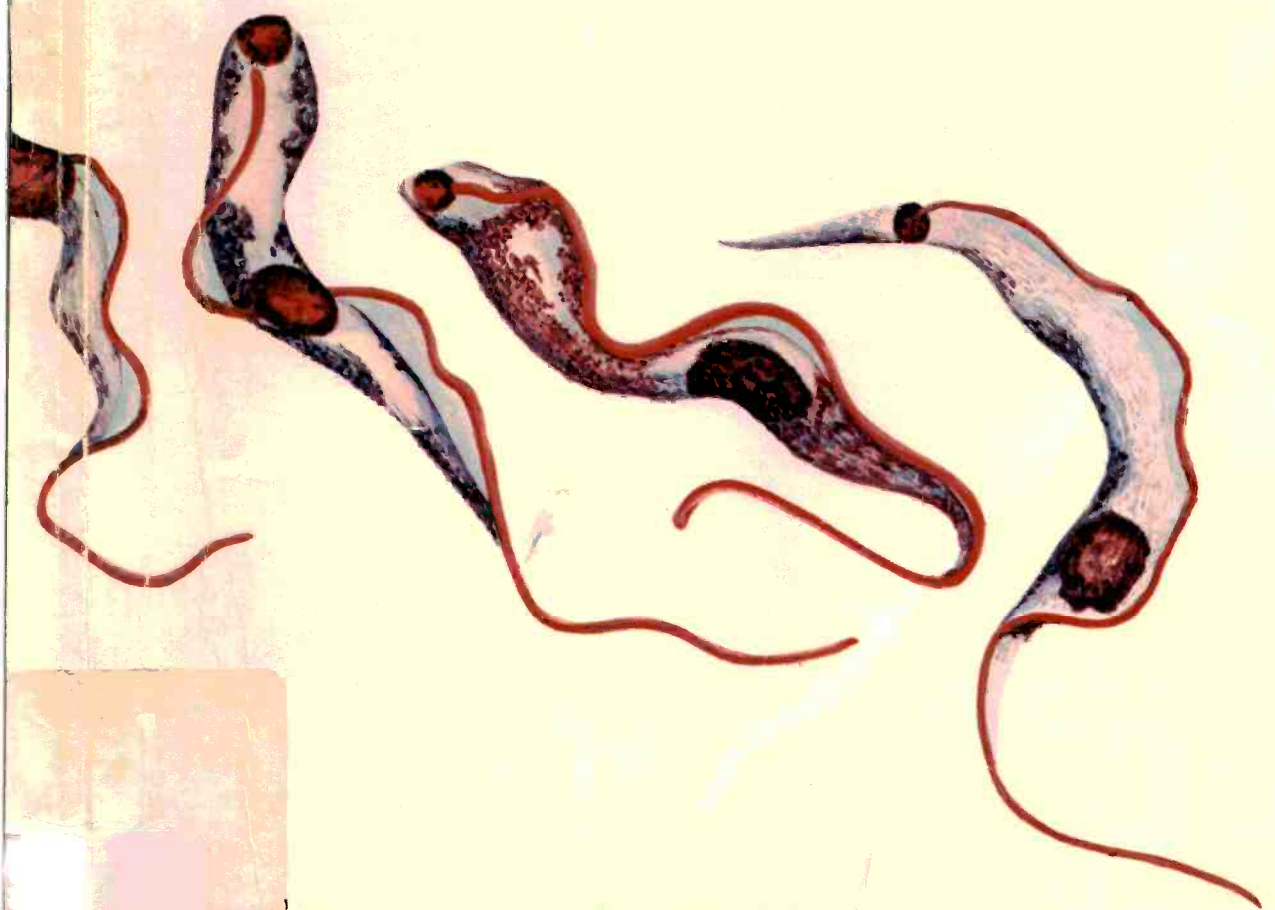
IDRC-132e

Pathogenicity of Trypanosomes

Proceedings of a workshop held at Nairobi, Kenya, 20-23 November 1978



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Editors: **George Losos[1] and Amy Chouinard[2]**

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Contents

Pharmacologically active substances in *T. vivax* infections

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Abstract. We researched two pharmacologically active substances — bradykinin and serotonin — which are known to occur in animals with trypanosomiasis. Attempts were made to correlate the action of bradykinin and serotonin in vitro, with their actions in vivo in noninfected animals. Then, we experimentally infected heifers and goats with *T. vivax* and monitored the animals' physiologic changes (heart frequency, rectal temperature, ruminal motility) as well as their levels of bradykinin and serotonin, but we did not detect any substantial correlation between them.

In our study, my colleagues and I at the Institute of Tropical and Protozoan Diseases and at the Institute of Veterinary Pharmacology and Toxicology in the Netherlands experimentally infected animals with a mouse-infective *T. vivax* strain Y58, described by Leeflang, Buys, and Blotkamp (1976), and attempted to link the animals' changes in blood serotonin levels and bradykinin activity with the observed clinical and pathological changes. We also compared dose-dependent inhibition of rumen motility and possible change of heart rate (caused by intravenous administration of bradykinin and serotonin) with the blood levels of serotonin and bradykinin activity and the observed clinical and pathological changes in our infected animals.

Bradykinin

Earlier studies have shown that bradykinin (BK) and its natural derivatives kallidin (Lys-BK) and Met-Lys-BK cause:

- Contraction of the nonvascular smooth muscles (bronchi, stomach, intestines, uterus),
- Increased capillary permeability (Lewis 1970),
- Lowered blood pressure due to arteriolar, capillary, and venular dilation, and tachycardia, which is probably caused by a reflex mechanism (Trautschold 1970),
- Relaxation of the rat's duodenum in vitro (Horton 1959; Gaddum and Horton 1959),
- Pain (when injected intradermally) (Armstrong 1970),
- Migration of leukocytes (Lewis 1962), and
- Tachyphylaxis (following repeated administrations) (Armstrong 1970; Collier 1970).

We undertook studies of bradykinin on ruminal strip smooth muscle tone and on ruminal contractions. In vitro (0.2 ng/ml) it caused a ruminal strip preparation to contract, and in vivo (0.5μg/kg intravenously injected into goats) it caused an inhibition of the reticuloruminal contractions (Van Miert 1970; Veenendaal unpublished data). These results may reflect an enhanced reticuloruminal smooth muscle tone causing reflex inhibition of the normal cyclical movements. Reflex inhibition occurs when the high-threshold tension receptors in the reticuloruminal wall, innervated by vagal sensory fibres, are tonically (continuously) active (Leek 1969).

How *T. vivax* activates bradykinin release is not clear; however, elevated levels of the substance are found very early in infection. In the early stages at least, therefore, its activity is probably not due to immune complexes, as suggested by Boreham and Wright (1976a). It may be, however, that immune complexes are responsible for elevated levels that can be observed later. Boreham and Wright (1976a) did not exclude the possibility that the trypano-

somes trigger the bradykinin activity; this view gains some support from work in which Seed (1969) and Tizard and Holmes (1977) were able to derive vasoactive materials from trypanosomes.

In cattle we infected with *T. vivax*, we found the highest level of bradykinin (60 ng/ml blood) at 4 days post infection. Levels gradually declined after that until about day 20, when an increase was noted and a rise–fall pattern began to emerge (Van den Ingh, Zwart, Van Miert et al. 1976; Van den Ingh, Zwart, Schotman et al. 1976).

When we attempted to correlate these findings with pathological and clinical changes that have been attributed to bradykinin activity and/or trypanosomiasis, our results were inconclusive.

Our studies of BK had suggested that uninfected animals experience inhibition of ruminal motility during increased bradykinin activity, but our studies in the *T. vivax*-infected animals did not bear this out. We observed no correlation between a rise in bradykinin levels, fever, and decreased ruminal motility (Veenendaal et al. 1976), and the literature on trypanosomiases contains no suggestion of greatly decreased appetite. Shien et al. (1975) observed that dogs infected with *T. evansi* maintained a good appetite even during periods of high parasitemia and fever. Similar observations were made by Mamo and Holmes (1975) in cattle infected with *T. congolense*. In general, gastric secretion and hunger contractions (reticuloruminal contractions in ruminants) are absent in animals with fever and anorexia is present as long as the fever persists (Van Miert in press).

Similarly, we were unable to correlate raised bradykinin levels with increased capillary permeability, although the condition has been noted in studies of both bradykinin and trypanosomiasis. At any rate, there are many factors that may contribute to vascular leakage in trypanosomiasis, only one of which is bradykinin.

For instance, vasoactive amines, such as histamine and serotonin, are likely chemical mediators of vascular leakage. In addition, fibrinopeptides, which are liberated from fibrinogen molecules and certain fragments of the complement system, i.e., C3a and C5a, are known to induce vascular leakage (Ryan 1974; Eisen and Vogt 1970). In trypanosomal infections, fibrinopeptides may be produced by disseminated intravascular coagulation or by complement, activated by trypanosomes. The latter possibility gains some support from studies of Musoke and Barbet (1977) who have described activation of the complement system by a variant antigen of trypanosomes. Another possibility is that the platelets or fibrinothrombi formed during trypanosomal infections directly affect the vessel wall and that they cause anoxia of the cells and tissues, resulting in increased vascular permeability.

Free fatty acids and reduction of lysophosphatidyl choline have also been mentioned as a cause of vasopermeability, but their role in vivo is not clear (Tizard and Holmes 1976; Roberts and Clarkson 1977). Another possibility is the low albumin content or heart failure in trypanosomiasis.

We would, therefore, hesitate to consider the observed vascular permeability in our animals and laboratory studies as only due to bradykinin, although a local effect of the substance cannot be excluded.

The story was much the same for blood pressure changes and tachycardia. In *T. vivax*-infected goats, there was no correlation between bradykinin levels and pulse rate, although this finding does not rule out hypotension. For example, in *T. brucei*-infected rabbits, Wright and Boreham (1977) found hypotension without increased heart rate.

The poor, or nonexistent, correlations between the clinical signs of *T. vivax* and the pattern of bradykinin levels are puzzling and cannot be explained in terms of tachyphylaxis. In an attempt to do so, we injected goats intravenously four times with bradykinin (2 μg/kg body weight) at 30-minute intervals before and after infection with *T. vivax*; there was no marked difference in response.

Serotonin

Some of the effects that have been observed after intravenous injection of bradykinin can also be found after vast intravenous injection of serotonin. Serotonin is another pharmacologically active substance formed during trypanosomiasis. It causes lowered blood pressure accompanied by tachycardia, dilatation of the vascular bed, and contraction of the main vascular smooth muscles (intestines, stomach, and bronchi). Tachyphylaxis, too, is common after repeated serotonin infusions (Szabuniewicz and McCrady 1977). Our work in goats showed serotonin decreased rumen motility but did not affect heart rate.

Little is known about the mechanism that activates serotonin during trypanosomiasis. It is known that serotonin is produced by several cells but not the thrombocytes, which only store the substance. When aggregation occurs, thrombocytes are destroyed, and they release the serotonin, which is either broken down very rapidly or taken up again by various other cells. Thus the serotonin in whole blood is a measure of thrombocyte destruc-

tion: when the thrombocytes are destroyed, blood serotonin levels drop.

During trypanosomal infections, the thrombocytes may be lowered by intravascular platelet aggregation or by disseminated intravascular coagulation. Studies to date suggest that both mechanisms operate but that one of them assumes a dominant role. For example, we found that thrombi consisted mainly of fibrin (Van Dijk, Zwart, and Leeflang 1973) in acute *T. simiae* infections and in the late stages of *T. vivax* infection in mice, whereas mainly platelet aggregation was found in goats dying during the acute stage of the infection (Van den Ingh et al. 1976a,b). Platelet aggregation also occurs in surviving animals, although the pathologic effects of it seem to depend largely on whether the reticuloendothelial system (RES) is able to remove the aggregations from the circulation (Van den Ingh et al. 1976a,b).

The fall in blood serotonin during temperature peaks, which are associated with peaks of parasitemia, and the presence of many platelet-thrombi in goats dying during overwhelming parasitemia suggested to us a correlation between *T. vivax*, parasitemia, platelet aggregation, blood serotonin decrease, and fever (Veenendaal et al. 1976, Van den Ingh et al. 1976a,b). Our studies suggested that *T. vivax*, either dead or alive, did not directly affect goat thrombocytes but immune complexes of *T. vivax* and antibody induced serotonin release (Slots et al. 1977). The serotonin released from platelets is rapidly destroyed or taken up by certain cells in vivo, and whole blood serotonin is a valuable parameter of thrombocyte destruction. We believe a systematic effect is unlikely, however. When we infused two normal goats with 0.5 μg serotonin/kg bodyweight for 300 minutes, which approximates the serotonin released during a thrombocytic crisis in trypanosomiasis, there was a slight increase in ruminal motility and in the breakdown product of serotonin, 5-hydroxy indoleactic acid (5-HIAA) in the urine; the latter, however, was not found in *T. vivax*-infected goats. This may be an indication that the uptake of released serotonin plays a role during *T. vivax* infections.

These experiments do not exclude the possibility of a local effect of serotonin, e.g., in the lungs, during trypanosomiasis.

Conclusions

It should be realized that release of mediators, complement activation, platelet aggregation, blood clotting, and tissue damage are all interrelated (Eisen and Vogt 1970; Ryan 1974). Most investigators have studied only a single component of these interrelated reactions during trypanosomiasis. Very little is known about how the different mediators potentiate or mitigate each other or about the role of the trypanosomes themselves. Moreover, the in vitro activities of these mediators may differ markedly from their effects on living animals, and their local effects may not be systemic.

How little we know about this ability to modify the chain of reactions is demonstrated by our studies of flurbiprofen, a potent anti-inflammatory and antipyretic agent, which inhibits platelet aggregation induced in vitro by epinephrine, collagen, and thrombin. This drug inhibited the febrile reactions in *T. vivax*-infected goats but did not prevent or reverse the associated drop in blood serotonin level. Moreover, it was apparent that flurbiprofen had a deleterious effect: all the goats died with a disseminated intravascular coagulation found at postmortem examination (Van Miert et al. 1978).