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Responsiveness of the rat vas deferens to catecholamines and electrical stimulation during an infection with *Trypanosoma brucei*

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Summary

The effect of an acute infection with *Trypanosoma brucei* on the reactivity of the rat vas deferens to noradrenaline, tyramine and field stimulation was studied. The vas deferens isolated from infected animals was more responsive to field stimulation than control rat vas deferens. The effect was more noticeable at lower frequencies of stimulation suggesting an increased sympathetic discharge from presynaptic nerve endings. Also both noradrenaline and tyramine were more effective on the infected vas deferens suggesting a postjunctional sensitization of α adreno ceptors. These results are explained in terms of the pharmacologically active substances released during an acute infection with *T. brucei*.

Résumé

L'effet d'une contagion sévère avec le *Trypanosoma brucei* sur la réaction du rat vas deferens à la noradrénaline, la tyramine et la stimulation éléctrique a été étudié. Le vas deferens, enlevé des animaux infectés s'averè plus sensible à une stimulation éléctrique que celui du rat du contrôle. L'effet était plus perceptible à basse fréquence de stimulation éléctrique, ce qui suggère un dégagement élevé et sympathique des nerfs périphériques présynoptiques. Egalement, la noradrénaline et la tyramine étaient plus efficace sur la vas infecté. Ceci suggère une sensibilisation post-jonctionale des α adrénocepteurs. Ces résultats sont expliqués en fonction des substances pharmacologiquement actives dégagées au moment d'une infection avec *T. brucei*.

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Introduction

Vascular lesions are associated with *T. brucei* infections in laboratory rodents. An irregular constriction of the central ear artery of the rabbit has been observed during an infection with *T. brucei* (Goodwin & Hooks, 1968). However intra-arterial injection of α-adrenoceptor antagonist, phenoxybenzamine, produced a dilatation of the constricted artery (Goodwin & Hooks, 1968) thus suggesting either an increased sympathetic discharge or increased sensitivity of the blood vessels to circulating catecholamines as contributing to the vasoconstriction. However, phenoxybenzamine is not too specific an α-adrenoceptor antagonist. For example, histamine constricts the rabbit central ear artery which is antagonized by phenoxybenzamine (Bamgbose & Okpako, 1974) revealing a vasodepressor effect. Histamine release has been reported to take place during an infection with *T. brucei* (Richards, 1965). Thus, histamine could directly or indirectly contribute to the vasoconstriction. Greer, Cain and Schotellius (1979) have demonstrated a significant increase in active tension (measured by the reactivity to K^+) of the rat isolated caudal artery during an infection with *T. brucei*.

In this investigation, we have studied the responses of the rat isolated vas deferens to noradrenaline, tyramine and electrical stimulation during an infection with *T. brucei* as a prelude to a detailed study of α-adrenoceptor characteristics of the vas deferens during an infection with *T. brucei*.

Materials and methods

Adult male albino rats (Wistar strain) weighing between 170 and 200 g were used in this study.

Infection of rats

Blood was collected from heavily infected rats and diluted to about 1.0 ml. Rats were then injected intraperitoneally with 0.4 ml of this suspension (containing approximately 3×10^{-6} parasites). The parasitaemia was followed daily by examining freshly prepared blood films. The animals were sacrificed when the parasitaemia was greater than 75% which usually occurred at about the 3rd day post-infection.

Isolated tissue preparation

Each rat was killed by a sharp blow to the head and bled through a cut in the carotid artery. The abdominal cavity was opened up and the testes were pushed out from the scrotal sacs. The exposed vasa deferentia were carefully desheathed and dissected out. Each vas deferens was suspended in a 20.0 ml organ bath containing Tyrode solution of the following composition: NaCl 137; KCl 2.7; $MgCl_2$ 0.9; NaH_2PO_4 0.3; $CaCl_2$ 1.8; $NaHCO_3$ 11.9 and glucose 5.6 mmol/l. The solution was maintained at 37°C and was continuously bubbled with air. The tissue was allowed to stabilize (under a resting tension of 0.5 g) for 60 min during which the Tyrode solution was replaced at 15–20-min intervals. Isotonic contractions (magnification: ×7) were recorded through a frontal writing lever on smoked paper. For field stimulation the vas deferens was passed through a platinum ring electrode. Stimulation parameters were as indicated in the text.

Drug solution

(−) Noradrenaline (BDH) was dissolved in distilled water with an equivalent amount of sodium metabisulphite to prevent oxidation. Tyramine hydrochloride (BDH) was also dissolved in distilled water. Fresh dilutions of the drugs were made each day of the experiment.

Statistical analysis

Where appropriate, mean values were compared using the Student's *t*-test. The difference between mean values were assumed to be significant when $P<0.05$.

Results

In preliminary experiments, it was observed that KCl generated greater tension that either of the sympathomimetics tested — i.e. noradrenaline (NA) and tyramine. Therefore, measurements of contractile responses to the sympathomimetics were expressed relative to the highest response to KCl (in many cases, maximum contractions to KCl were not attainable). Noradrenaline (1.95×10^{-6}–6.24×10^{-5} M) concentration-dependently contracted the vas deferens from both infected (IVD) and uninfected (i.e. control = CVD) rats. In all cases, the threshold concentration of NA required to produce a contraction ranged from 9.9×10^{-7} M to 1.95×10^{-6} M. The IVD was more responsive to NA than CVD. As shown in Fig. 1a, the difference in responsiveness was significant ($P < 0.05$) at all concentrations of NA. The mean $-\log EC_{50}$ values were 5.05 ± 0.15 and 4.70 ± 0.10 on the IVD and CVD, respectively, thus showing that the IVD was approximately twice as responsive to NA as CVD. However, the $-\log EC_{50}$ values were not significantly different ($P > 0.05$) from each other. Tyramine (7.7×10^{-6}–1.2×10^{-4} M) also reproducibly and concentration-dependently contracted the vas deferens from both infected and non-infected rats. Figure 1b shows that the IVD was significantly ($P < 0.05$) more responsive than CVD at all concentrations of tyramine. Mean $-\log EC_{50}$ values were 4.50 ± 0.08 and 4.20 ± 0.10 on IVD and CVD, respectively, showing an approximate 2-fold increase in responsiveness even though the $-\log EC_{50}$ values were not significantly different ($P > 0.05$). Field stimulation (50 V, 1 msec, 30 sec) of the vas deferens produced frequency-dependent contractions. The IVD was more responsive than CVD at the lower but not at the higher frequencies of stimulation (Fig. 2). At the higher frequencies of stimulation, there was a slight though insignificant ($P > 0.05$) reduction in the responsiveness of the IVD to field stimulation.

Discussion

These results show that there is an increased responsiveness of the rat vas deferens to noradrenaline, tyramine and field stimulation (at lower frequencies of stimulation) during an

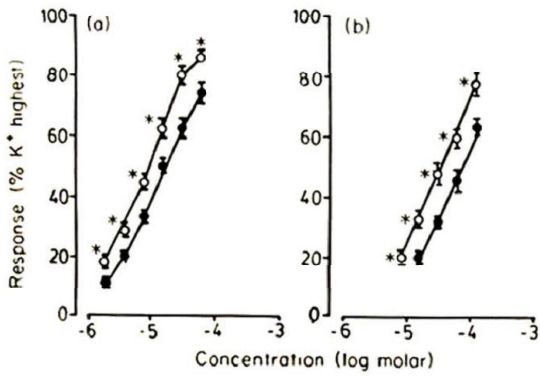

Fig. 1. Responsiveness of the vas deferens isolated from normal (uninfected) rats (●) and rats infected with *T. brucei* (approx. 3×10^{6} parasites) (○), to (a) noradrenaline and (b) tyramine. Note that the vas deferens isolated from infected rats was significant ($P< 0.05$) more responsive to noradrenaline and tyramine at all concentrations.

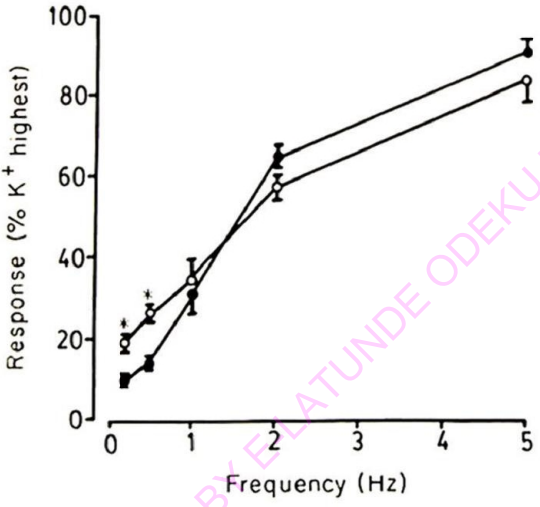

Fig. 2. Responsiveness of the vas deferens isolated from control rats (●) and rats infected with *T. brucei* (approx. 3×10^{6} parasites) (○) to field stimulation (50 V, 1 msec, 30 sec). The responses are only significantly different ($P < 0.05$) at the lower frequencies of stimulation.

infection with *T. brucei*. An underestimate of the responsiveness of the IVD is not unlikely in this investigation. Because K^+ generated greater tension than any of the sympathomimetics, we have in this study expressed all contractile responses as a percentage of the highest response to K^+. However, we have consistently observed (Adeboye & Oriowo, unpublished data) as have Greer *et al.* (1979) on the rat isolated caudal artery, that the IVD was more responsive to K^+ than CVD. Thus, whatever was gained in terms of responsiveness was lost while relating to the highest K^+-induced contractions. This underestimate of the responsiveness of the IVD could therefore account for the lack of significant difference in the $-\log EC_{50}$ values for NA and tyramine on the IVD and CVD, respectively. The factors responsible for the increased responsiveness are unknown. It is certainly not due to a defective uptake mechanism which would allow NA to accumulate in the synaptic cleft. This is because tyramine is an indirectly acting sympathomimetic amine (Axelrod *et al.*, 1962) and is a substrate for the uptake mechanism (Iversen, 1967). Tyramine would therefore not be as responsive on the IVD if the uptake mechanism were defective. *T. brucei* infection in rodents is accompanied by an increased release of pharmacologically active substances including histamine and kinins (Goodwin & Richards, 1960; Richards, 1965). Bradykinin contracts the rat vas deferens with a threshold at about 5×10^{-8} M (Negri, Erspamer & Piccinelli, 1973) which is less concentration of kinins in the plasma during an infection (Richards, 1965). With the recording device used in this study, it is not known whether or not there was a change in the basal tone of the vas deferens. It is possible that the increased tissue concentration of the kinins enhanced the response of the vas deferens to agonists *in vitro* since it has been shown that sub-threshold concentration of contractile agonists could enhance the effect of other agonists (De la Lande, Canwell & Waterson, 1966; Asano & Hidaka, 1980). However, kinins are vasodilators and so could not have been responsible for the vasoconstriction in the ear artery (Goodwin & Hook, 1968) and the increased active tension in the caudal artery of the rat (Greer *et al.*, 1979). Though histamine constricts the central ear artery of the rabbit (Bamgbose & Okpako, 1974), it does not contract the vas deferens (Vohra, 1981) and actually inhibits the response of the vas deferens to field stimulation (Vohra, 1981). Increase in histamine release would therefore not explain the increased responsiveness of the vas deferens during the infection with *T. brucei*. It is therefore likely that separate mechanisms

could explain the increase in reactivity of vascular and non-vascular tissues during the infection. Infections are generally stressful conditions and stress leads to increased release of hydrocortisone. In the rat, corticosterone and not hydrocortisone is the major glucocorticoid. Corticosterone has been shown to enhance the responses of the rat anococcygeus muscle to agonists *in vitro* (Gibson & Pollock, 1975; Gibson, Pollock & Spence, 1976) as well as to electrical stimulation (Gibson *et al.*, 1976).

The effect on nerve stimulation is more significant at low frequencies of stimulation (Gibson *et al.*, 1976). Our results on the field-stimulated vas deferens are very similar to these observations. We therefore suggest that the increased responsiveness of the rat vas deferens during an infection with *T. brucei* may be due to an increased output of corticosteriods as a result of stress imposed by the infection. This hypothesis would require confirmation by actual measurements of corticosteroids in the plasma (probably in tissues too) during an infection with *T. brucei*.

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