Acta Medica et Biologica
Vol. 46, No. 1, 7-10, 1998

Effects of Heat-killed *E. coli* Pretreatment on Agonist-induced Contractile Response of the Isolated Vas-deferens

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Received August 5 1996; accepted May 12 1997

Summary. **The effect of noradrenaline (NA), acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) on the contractility of the vas-deferens smooth muscle isolated from rats treated with heat-killed *E. coli* (endotoxin) was investigated. The contractile tension to these drugs was significantly decreased as compared with control values at different post-treatment time periods except for 5-HT at 48 h post treatment time. The 48 h tissues showed an increased sensitivity to 5-HT, while the same doses produced a decreased contractility in the 72 h tissues.**

The structural integrity of the vas deferens is vital in the ionic transport mechanism essential to Excetation-Contraction coupling. *E. coli* induced a decreased responsiveness of the vas deferens to these mediators probably due to the liberation of free radicals. The latter itself could cause damage to the oxidizable muscle membrane structures or by a direct action on the membrane enzyme systems. Either of these postulates will interfere with smooth muscle contractility and thus impede the passage of spermatozoa to the testes in the septic state.

Keywords—Heat-killed *E. coli*, endotoxin, vas-deferens, contractility.

INTRODUCTION

A large body of literature has conbined the inhibitory activity of the bacterial endotoxin on muscular activity *in vivo*.[1-4] Many clinical observations have suggested that bacterial infections affect the integrity and contractility of the smooth muscle.[1,3,5] The association of infections with a variety of functional disturbances of the muscular transport system and peristaltic activity has also been reported.[3,6,7] Infection-induced dysfunction of muscular transport system has been observed in the absence of underlying mechanical obstruction.[7,8] However, the underlying mechanisms and responses of the vas deferens to endogeneous mediators in the septic state have not been well defined. We therefore undertook a study to investigate the contractile responses of vas-deferens isolated from rats treated with heat-killed *E. coli* at 48 and 72 h post treatment periods to noradrenaline, acetylcholine and serotonin with the view to determining their effects on spermatozoa transport to the testes in septicemia.

MATERIALS AND METHODS

Endotoxemia

The method described by King and Cox[10] was used to induce endotoxemia. *E. coli* was grown in a liquid culture media (from the Department of Medical Microbiology, College of Medicine University of Lagos) and standardized by correlating optical densities of bacterial suspension with quantitative colony count to determine the number of organisms per ml. The bacterial suspenison was then heated in a bath at 60℃ for 3 h to kill the *E. coli*. Bacterial death was confirmed by culture.

Animals

Inbred healthy male albino rats (from our Laboratory Animal Centre, College of Medicine University of

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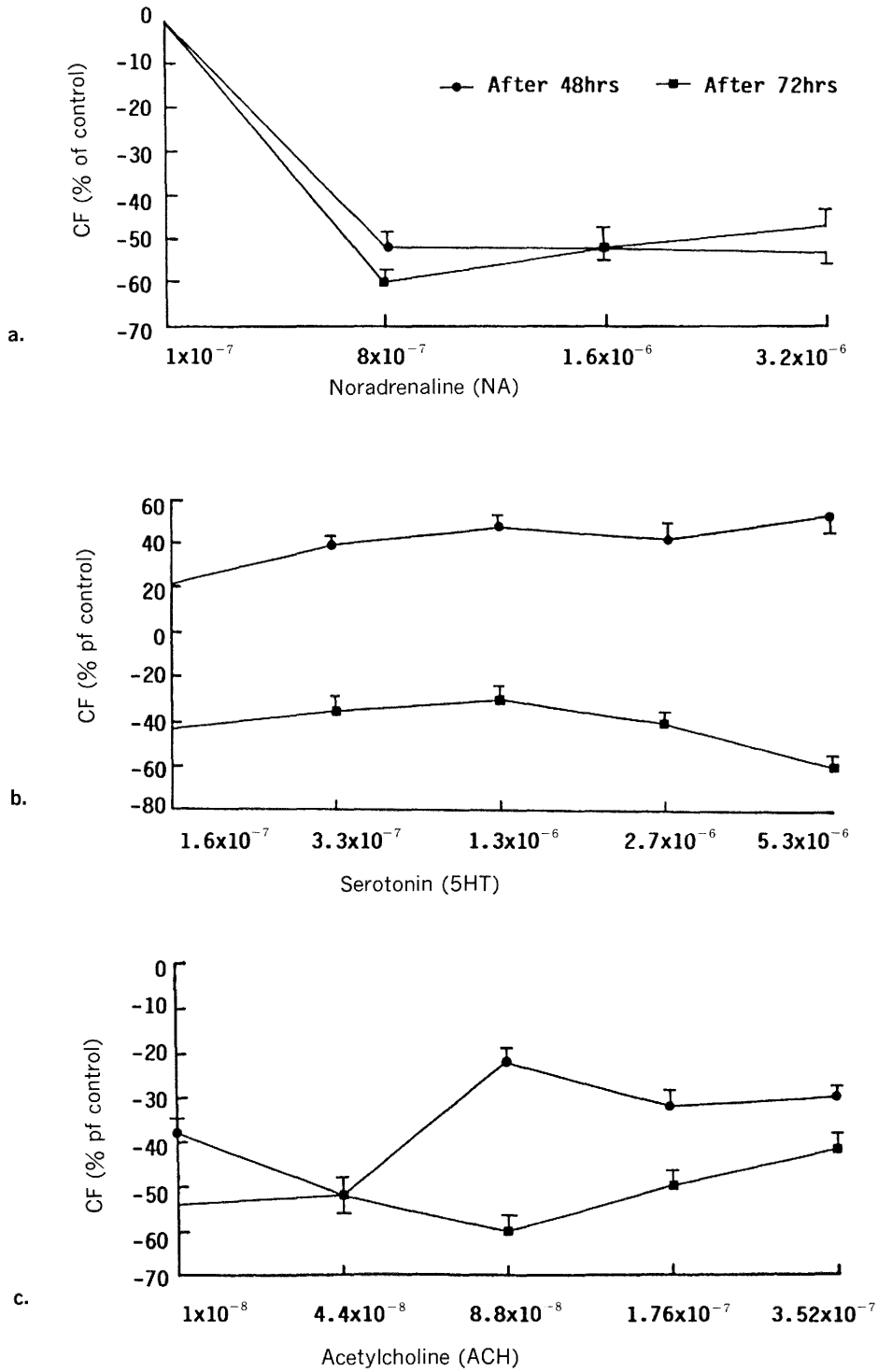

Fig. 1. Contractile Force (CF) of rat isolated Vas deferens smooth muscles (**a**) in the presence of various doses of NA, (**b**) 5HT and (**c**) ACH. Each point represents the mean±S.E. n, 10.

Lagos), weighing 250-300 g, were used. They had free access to both food and water and were divided into groups, viz, saline control and heat-killed *E. coli* treated groups.

Endotoxemia was induced in three groups of rats of the same body weight (n=10) by intraperitoneal (ip) injection of heat-killed *E. coli* (9.7×10^9/kg), which resulted in about 25% mortality. The rectal temperature was recorded in survivors with the aid of a clinical thermometer inserted into the rectum to a distance of 2 cm. Rises in temperature, diarrhoea and weight loss were used as indicators for endotoxemia.[3,10,11] The three control groups (n=5) were injected (ip) with equivalent volumes of isotonic saline.

Both the control and treated rats were killed at 48 and 72 h post-treatment by decapitation. The vas-deferens (approximate length 2.0 cm) was removed free of connective tissue and immersed in an organ bath containing Krebs' solution bubbled with 95% O_2 and 5% CO_2, maintained at a constant temperature of 37℃. A resting tension of 1 g was applied to the tissue and allowed to equilibrate for 1 h before the experiment commenced. Drugs were added in a cumulative manner. For each concentration of the drug, results have been calculated at the time of maximum effect expressed either as the mean contractile force or percentage change from the control values from untreated rats. Data are expressed as means±S.E. 5-hydroxytryptamine (5HT), noradrenaline (NA) and acetylcholine (ACh) were obtained from Sigma Chemical Co (St. Louis, MO, USA). Drugs were dissolved and diluted in a physiological solution on each experimental day.

RESULTS

The contractile response of the vas-deferens isolated from endotoxemic rats to NA and ACh was significantly attenuated at both post-treatment periods. Response to 5-HT at 48 h post treatment was profoundly high but significantly reduced at 72 h post treatment. Within the range of NA concentration of 8×10^{-7} to 3.2×10^{-6} M, the magnitude of contratile force produced in the vas-deferens of the treated rats was reduced by 52±4.1 and 60±6.1% at 48 and 72 h post treatment respectively, when compared with the control ($p<0.05$). The minimum effective concentration that elicited a measurable response in the vas-deferens from treated rats was several times greater than that obtained in control rats (Fig. 1A). On the other hand, 5HT at the dose range of 1.6×10^{-7} to 5.3×10^{-6} M in the 48 h tissues produced a significant increase by 54±6.4% in the amplitude of contraction above the control values. The 72 h tissues responded to 5-HT with a reduction the same as that produced by NA at the same time period (Fig. 1B). Ach (1×10^{-8} to 3.52×10^{-7} M) did not produce any alteration in the pattern of response. The contratile force was significantly reduced by 60±5.1% in the 72 h tissues ($p<0.05$) (Fig. 1C).

DISCUSSION

Administration of heat-killed *E. coli* to rats resulted in decreased contractile responsiveness of the vas-deferens smooth muscle to NA, 5-HT and Ach. These altered responses became evident 48 h post tratment, particularly those to NA and ACh. During the same time period, the tissues showed increased sensitivity to 5-HT. However, in the 72 h tissues, the responses were significantly reduced. King and Cox[9] reported that endotoxin activity is evident only upon its liberation from bacterial cell-wall following cell lysis or death. Similarly, in this investigation we have used killed *E. coli* of a high concentration and have observed significant signs of endotoxemia and depressed vas-deferens smooth muscle contratility to the test drugs. Teague and Boyarsky[7] demonstrated an inhibitory effect of endotoxin on ureteral peristalsis in intact dogs, though not to these mediators. Conversely, Malin[12] observed a stimulatory effect with lower concentratoins of endotoxin on canine ureteral strips. Although the mode of action of endotoxin at the cellular level is not well defined, Diplock and Lucy[1] showed that free radicals are liberated from the liver during septic shock, and they induced the destruction of oxidizable membrane structures which eventually lead to cell damage. Similarly, Tenny and Raffer[13] have suggested that endotoxin may disrupt cell membrane enzyme systems or ionic transport mechanisms vital to muscular contractility. This suggests that endotoxin could depress the muscular transport system *in vivo* by either of the two mechanisms. Thus, the deleterious attenuating effect of endotoxin on the basal smooth muscle contractility could possibly accunt for the observed decreased responsiveness to these endogenous mediators.

CONCLUSION

The mechanism by which endotoxin produced this effect is not known, but the clinical significance of our finding is the potential risk of consequences of post-infective infertility which may result from depressed muscular transport of motile sperm to the testes.

Acknowledgments. This study was funded by the College of Medicine, University of Lagos. We are grateful to Miss C Zambezi for secretarial assistance.

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