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THE INHIBITORY MATERIAL IN EXTRACTS FROM THE BOVINE RETRACTOR PENIS MUSCLE IS NOT AN ADENINE NUCLEOTIDE

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Methods are described for the removal of adenosine or purine nucleotides from extracts of bovine retractor penis muscle (BRP). These methods did not interfere with the biological test preparations. Removal of adenosine and purine nucleotides by these methods did not modify the inhibitory action of the extract on the BRP. The effect of the extract on the BRP resembles that of inhibitory nerve stimulation. If the inhibitory substance present in the extract is the inhibitory transmitter, then the results indicate that, in this tissue, the transmitter is neither adenosine nor a purine nucleotide.

Introduction

The rat anococcygeus and the bovine retractor penis (a continuation of the anococcygeus in the ox) each possess a motor adrenergic innervation and an inhibitory innervation whose transmitter is unknown (Gillespie, 1972; Klinge & Sjöstrand, 1974; Ambache, Killick & Zar, 1975; Burnstock, Cocks & Crowe, 1978). Extracts of either muscle powerfully inhibit both muscles and thus may contain an inhibitory transmitter (Ambache *et al.*, 1975; Gillespie & Martin, 1978) or at least a substance involved in the inhibitory mechanism. Burnstock and his coworkers (for a review, see Burnstock, 1972) have presented evidence that in many tissues the transmitter at non-adrenergic, non-cholinergic nerve endings is adenosine triphosphate (ATP) or adenosine, and they have coined the term 'purinergic' to describe the nerve fibres. We have found that extracts of the bovine retractor penis contain large quantities of adenosine or purine nucleotides. This paper describes two methods for the removal of these compounds without interfering with the biological test preparations and which, therefore, allow us to test whether adenosine or purine nucleotides are responsible for the inhibitory effect.

Methods and Results

BRP muscle was extracted with cold methanol, the extract evaporated to dryness, resuspended in isotonic saline, activated with acid (Gillespie & Martin, 1978) and tested on the BRP and on the blood pressure of the rat, anaesthetized with pentobarbitone. The extract inhibited the BRP and produced a biphasic effect on the rat blood pressure, a fall followed by a rise. A portion of the methanol extract was passed over a strong anion exchange resin, Biorad AG1-X8, the column washed with distilled water then eluted with 500 mM NaCl solution and the eluate, after dilution to isotonicity with distilled water, tested for activity. On the BRP the inhibitory effect was unaltered but on the rat blood pressure the pressor effect was lost; the extract now produced a pure depressor response (Figure 1a), which was unaffected by atropine in doses abolishing the effect of a matching depressor dose of acetylcholine. The first indication that inhibition of the BRP and the depressor effect on the rat blood pressure were not due to the same material came from the observation that placing the extract for 2 min in a boiling water bath abolished the inhibitory response on the BRP completely without reducing the depressor effect on the rat blood pressure. This depressor effect could be matched by injecting the same volume of a solution of either ATP or ADP in the concentration range 50 to 450 μg/ml. Solutions of ATP and the tissue extracts absorbed u.v. light with identical absorption spectra peaking at 260 nm, and the degree of absorption by the tissue extracts suggested the same ranges of nucleotide concentration as the rat assays. Two methods were used to remove these nucleotides. In the first the extracts were treated with the enzyme apyrase followed by 5′ nucleotidase to convert the nucleotides to adenosine and phosphate. The extract was then passed through a cationic resin, AG50W in the Na^+ form which retained the adenosine. The second method was to bind the adenine nucleotides to alumina at pH 9.0. Both methods abolished u.v. absorption at 260 nm and the depressor effect on the rat blood pressure. Neither reduced the inhibitory effect on the BRP (Figure 1b).

Discussion

The present results show conclusively that the inhibition of bovine retractor penis muscle by an extract from that muscle is not due to adenosine or a purine nucleotide.

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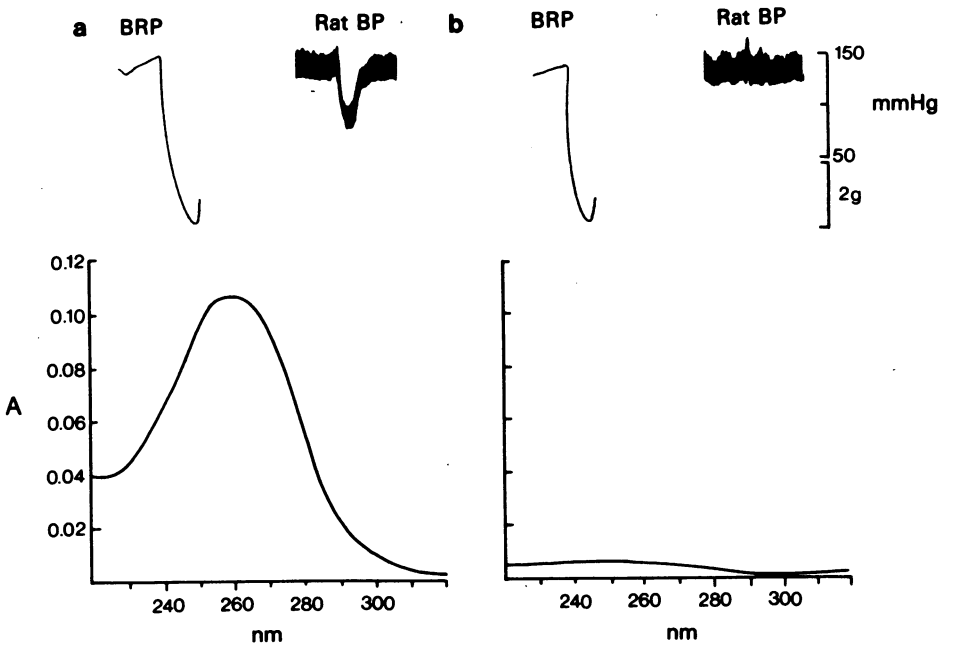

Figure 1 The upper records show the effect of inhibitory extract from the bovine retractor penis muscle (BRP) on the BRP muscle and on the blood pressure of the pentobarbitone-anaesthetized rat (BP). The graphs in the lower part of the figure show the spectrophotometric absorbance of these extracts. The records in (a) were obtained from an extract which contained ATP or other adenine nucleotides as shown by the absorbance at 260 nm. This material inhibited the BRP and lowered the rat BP. Panel (b) shows the effects of the same extract after removal of these nucleotides by adsorption on alumina. The absorption peak at 260 nm has disappeared and the extract has no longer any depressor effect on the rat BP. The inhibitory effect on the BRP, however, is unaltered.

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