EXTRACTION FROM OX RETRACTOR PENIS OF AN INHIBITORY SUBSTANCE WHICH MIMICS ITS ATROPINE-RESISTANT NEUROGENIC RELAXATION

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The inhibitory post-ganglionic transmission in the retractor penis of the ox resembles that of the dog and is not cholinergic or adrenergic. Acid extracts of this tissue have yielded an unidentified, labile, inhibitory substance which mimics the effect of its inhibitory nerves.

Introduction

The retractor penis (R.P.) of the ox is known to be free from skeletal fibres and from ganglion-cells (Klinge, Pohto & Solatunturi, 1970). We find that it has the advantage, over that of other species (pig, sheep, horse etc.), of developing tone spontaneously in Krebs-Henseleit solution containing phentolamine, without requiring drug-induced contraction (by guanethidine or by spasmogens) to reveal the effect of its inhibitory innervation. Our results also show that this smooth muscle contains post-ganglionic inhibitory nerves with atropine-resistant transmission, which resemble the parasympathetic sacral innervation of the dog R.P., first studied by Langley & Anderson (1895) and shown by Luduena & Grigas (1966; 1972) to be non-cholinergic and not purinergic. Extracts of the R.P. tissue contain an unidentified, labile, inhibitory substance which could account for this type of transmission.

Methods

Isolated preparations. Steer R.P., obtained from various abattoirs, were transported at 0° C. Strips, 30 x 2 x 2mm, were dissected longtitudinally, usually from the middle third of the R.P. and suspended at 35° C in Krebs-Henseleit solution (mM: NaCl 112.9, KCl 4.69, $CaCl_2.2H_20$ 2.52, KH_2PO_4 1.18, $MgSO_4.7H_2O$ 1.1 or 1.5, $NaHCO_3$ 25.0 and glucose 11.1) containing phentolamine, 5.3 μM. Contractions were recorded isometrically via transducers at a load of 2 grams The preparations were stimulated transmurally at a pulse width of 1 ms, usually with single pulses at 1-2 min intervals. Some preparations, left over night in the organ bath at room temperature, could be used again at 35° C next morning, when they often appeared to be more sensitive to extracts. Drug concentrations are expressed as their respective salts.

Extraction of inhibitory substance(s). Two to six hours after death, 1 g pieces of R.P. from the proximal or middle third were solidified in liquid N_2, hammered in N_2-cooled, stainless steel or PTFE pulverizers, ground with disodium edetate (EDTA)-washed Pyrex fragments in ice-cooled Pyrex mortars containing 0.17 or 0.33 N HCl ('Aristar' in distilled water, deionized through Bio-Rad resin AG 501-X8), 1 ml/100 or 200 mg tissue, and centrifuged (0° C, 16000 *g*, 10 minutes). The ice-cooled supernatants, of pH 1-2, were titrated electrometrically to pH 6-7 with cold 'Aristar' 2 N NaOH or 'Analar' 1 N Na_2CO_3, sometimes in the presence of ca. 1/40 vol of sodium acetate buffer (0.2 M; pH 3), and were re-centrifuged, or allowed to sediment. Their inhibitory activity was determined immediately on the more sensitive of twin steer R.P. preparations in the presence of atropine, phentolamine propranolol and mepyramine.

Results

Pharmacological analysis of the inhibitory transmission. The isolated preparations at first lengthened; acetylcholine chloride, 1-10 μg/ml (cf. Klinge, 1970), 5-hydroxytryptamine creatinine sulphate, 10 μg/ml, histamine dihydrochloride, 1-10 μg/ml and adenosine 5'-triphosphate (ATP) disodium salt, 10 μg/ml, were motor.

After 0.5-4 h tone ($\geqslant$ 2 g) appeared, which was usually well maintained; a few preparations in which the tone level was not steady were discarded. As soon as tone developed, single 1 ms

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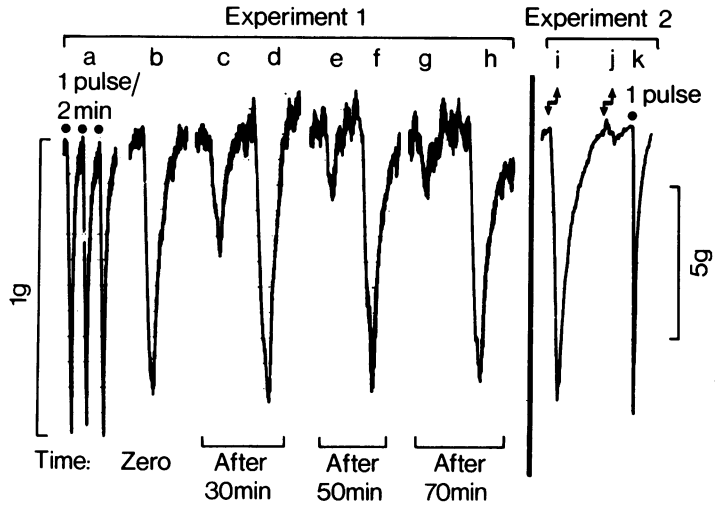

Figure 1 The inhibitory activity of steer retractor penis (R.P.) extracts decays at room temperature (b-h) and is destroyed by heat ((i-k), from another experiment). Both assays on tonic steer R.P. strips in phentolamine, 5.3 μM; mepyramine, 1.25 μM; propranolol, 6.8 μM for Expt. 1 and 13.6 μM for Expt. 2 and atropine, 1.44 μM for Expt. 1 and 2.88 μM for Expt. 2. Neurogenic relaxations elicited by single 1 ms pulses at the dots in (a) and (k) are matched by inhibitory responses to R.P. extracts, given for 1 minute. (b-h), Responses to a fixed dose, $\equiv$ 1 mg of tissue/ml, of an extract of pH 6.1: at (b), (d), (f) and (h), control sample of the extract kept on ice; at (c), (e) and (g), parallel sample kept at 23° C, showing decay after 30 (c), 50 (e) and 70 (g) minutes. (i and j), Thermolability of another R.P. extract, pH 6.6: $\equiv$ 2.5 mg of tissue/ml before (i) and after (j) heating to 100° C for 2 minutes.

pulses elicited large, tetrodotoxin-susceptible inhibitions which were non-cholinergic because they were not mimicked by acetylcholine nor influenced by physostigmine, atropine, dimethyltubocurarine or pentolinium. These inhibitory responses were unaffected by guanethidine, propranolol, mepyramine or burimamide; and were not mimicked by noradrenaline. adrenaline, dopamine, 5-hydroxytryptamine, various amino acids (glycine, alanine, glutamic acid, aspartic acid and γ-aminobutyric acid) or by ATP.

Inhibitory activity of R.P. extracts. Unlike parallel, cold reagent blanks, the steer R.P. extracts, in doses of $\equiv$ 1.5 mg tissue/ml, produced relaxations, sometimes quite marked (Figure 1), which mimicked the neurogenic inhibitions and persisted in tetrodotoxin, dimethyltubocurarine or burimamide. Partitioned at pH $<$ 2 with 2-10 vol of freshly distilled ether, this inhibitory activity remained in the aqueous phase and was therefore not due to prostaglandins.

The lability of this inhibitory activity was shown by the fact that it decayed (more rapidly at room temperature) and could be destroyed by 2-5 min boiling (Figure 1); presumably because of this lability several extracts have had poor activity.

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