The rat anococcygeus; a new, densely innervated smooth muscle preparation

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The ideal innervated smooth muscle preparation would be one consisting entirely of smooth muscle cells, these cells arranged in parallel bundles to form a thin sheet in which the problem of diffusion would be minimal. The muscle should be represented bilaterally so that control and experimental tissue can be obtained from the same animal. If such a preparation was densely innervated and both pre- and postganglionic fibres available for stimulation this would be an additional advantage.

The preparation to be described has many of these advantages. The anococcygeus muscles arise independently from one, or more usually two, upper coccygeal vertebrae. The muscles at their origin lie close to one another and behind the terminal colon. They pass caudally and ventrally to sweep round the lateral side of the colon and unite in a well defined ventral band in front of the colon about 0·5–1 cm short of the anal margin. Some fibres continue down the posterior surface of the colon. The two muscles are easily and quickly dissected out, are about 3 cm long by 0·5 cm broad at the broadest part but only 150–300 μm thick. The external nerve (a branch of the perineal) can be retained with the muscle. Histological sections prepared by the technique of Hillarp & Falck show a dense adrenergic terminal plexus diffusely spread throughout the muscle fibres.

The response of the muscle suspended in Krebs saline at 36°C has been examined. There is no spontaneous activity nor any resting tone. The muscle contracts to field electrical stimulation or to stimulation of the extrinsic nerves. Neither contraction is affected by hexamethonium in concentrations up to 3×10^{-5} M, both are blocked by phentolamine (10^{-6} M) or guanethidine (10^{-5} M). Guanethidine has no effect on the response to noradrenaline. Atropine (3×10^{-5} M) has no effect on the response to nerve or field stimulation. Noradrenaline in low concentrations (10^{-7} M) causes contraction blocked by phentolamine. Acetylcholine in low concentrations (3×10^{-7} M) causes contraction blocked by atropine. Isoprenaline in low doses of 10^{-7}–3×10^{-6} M has no effect, higher concentrations of 3×10^{-6}–3×10^{-5} M cause large contractions. These are unaffected by propranolol (3×10^{-5} M). All doses of isoprenaline, including those too small themselves to cause contraction, potentiate the response to noradrenaline. These results suggest that the smooth muscle cells are adrenergically innervated, have both α-adrenoceptors and muscarinic receptors, but no or few β-adrenoceptors.

An unexpected inhibitory response appears with field stimulation in the presence of guanethidine. This inhibition is seen only with large doses of guanethidine which themselves raise muscle tone. It is exaggerated if muscle tone is raised by noradrenaline. The origin and mechanism of this response are being investigated.

Pharmacological observations on the vas deferens of the mouse

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The vas deferens of the mouse, stripped of mesenteric investment, was suspended between parallel platinum wire electrodes immersed in Hukovic's solution (Hukovic,

1961) at 32°C, gassed with 5% CO_2 in O_2. Contractions were recorded on smoked paper via an isotonic frontal writing lever. The tension applied to the muscle was 300 mg. The stripped vas deferens appeared relatively insensitive to agonist drugs. Noradrenaline ($5{\cdot}9\times10^{-8}$ M to $1{\cdot}2\times10^{-4}$ M) produced only small contractions and increasing the concentration up to $5{\cdot}9\times10^{-4}$ M failed to elicit a response comparable to that obtained by transmural stimulation. Adrenaline ($6{\cdot}0\times10^{-6}$ to $6{\cdot}0\times10^{-5}$ M) or acetylcholine ($2{\cdot}8\times10^{-6}$ to $4{\cdot}4\times10^{-4}$ M) acted similarly. Dopamine ($2{\cdot}6\times10^{-5}$ to $3{\cdot}7\times10^{-3}$ M), 5-hydroxytryptamine ($4{\cdot}9\times10^{-6}$ to $1{\cdot}5\times10^{-4}$ M) or histamine ($6{\cdot}5\times10^{-7}$ to $2{\cdot}0\times10^{-4}$ M) failed to contract the preparation.

Responses to noradrenaline were not facilitated by the use of the MAO-inhibitor Iproniazid ($3{\cdot}6\times10^{-6}$ to $7{\cdot}2\times10^{-5}$ M), COMT-inhibitor, Pyrogallol ($1{\cdot}6\times10^{-5}$ M) or uptake$_1$ inhibitor desmethyl-imipramine ($11{\cdot}9\times10^{-7}$ to $3{\cdot}8\times10^{-6}$ M). The preparation contracted in response to transmural stimulation at 30 Hz with 0·3 ms rectangular pulses of supramaximal voltage applied for 10 s every 3·25 minutes. Despite the insensitivity of the preparation to exogenous noradrenaline, the involvement of noradrenergic nerve fibres in the responses to transmural stimulation is suggested by the inhibitory action of guanethidine ($8{\cdot}1\times10^{-8}$ to $2{\cdot}0\times10^{-6}$ M) on these responses, and by the observations that dexamphetamine would protect against, or reverse, guanethidine-induced inhibition. In addition, desmethylimipramine ($9{\cdot}4\times10^{-9}$ M) prevented the inhibitory action of guanethidine.

However, the responses of the vas deferens to transmural stimulation were also impaired by atropine ($1{\cdot}4\times10^{-6}$ to $5{\cdot}6\times10^{-6}$ M). Dexamphetamine ($1{\cdot}1\times10^{-5}$ M) antagonized this effect of atropine, although with the higher doses of atropine recovery after dexamphetamine was not 100%. The inhibitory action of atropine was markedly reduced by prior exposure of the vas deferens to dexamphetamine ($2{\cdot}7\times10^{-6}$ M) or desmethylimipramine ($9{\cdot}4\times10^{-8}$ M).

The relationship of log dose to percentage inhibition of responses to transmural stimulation are linear for both guanethidine and atropine and the regression lines are parallel. In the presence of DMI or dexamphetamine, the regression lines are moved significantly to the right and remain parallel.

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REFERENCE

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Neuromuscular actions of lignocaine and prilocaine

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Jindal & Patel (1965) reported that lignocaine has a curare-like action on rat phrenic nerve-diaphragm preparations. Katz (1965) found that prilocaine sometimes decreased and sometimes increased the twitch tension of muscles stimulated via the motor nerve. We have examined the conditions under which prilocaine increases twitch tension and have investigated the action of lignocaine under similar conditions.

Rat phrenic nerve-diaphragm preparations were set up in 100 ml Krebs solution at 37°C as described by Jones & Laity (1965). The phrenic nerves were stimulated by