observation that the induced α-adrenoceptors were insensitive to phenoxybenzamine. The tissues were re-exposed to phenoxybenzamine in the presence of methacholine (which would be expected to protect the muscarinic receptors, and maximally induce α-adrenoceptors). Responses to noradrenaline were only reduced in proportion to the responses to methacholine. This suggests that methacholine is temporarily uncovering or unblocking the phenoxybenzamine-inhibited α-adrenoceptors. A corollary is that phenoxybenzamine does not alkylate directly to the α-adrenoceptor. This phenomenon could not be duplicated in guinea-pigs from the U.K. since their vasa deferentia do not contract in response to noradrenaline.

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The effect of chlorpromazine on carbachol binding to muscarinic receptors in intestinal smooth muscle

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The binding of agonists to muscarinic receptors in strips of longitudinal muscle from guinea-pig small intestine, as measured by the inhibition of the binding of an affinity label for the muscarinic receptor, [^{3}H]-propylbenzilylcholine mustard ([^{3}H]-PrBCM), differs from that of antagonists. Whereas antagonist binding curves in general approximate well to those expected for a simple mass-action interaction, those for agonists show an apparent negative co-operativity (Young, 1974). Similar effects have been observed using a microsomal fraction (Burgen & Hiley, 1975). There is other evidence that the binding of agonists differs from that of antagonists. Exposure of muscle strips to distilled water prior to the usual 1 h preincubation in normal Krebs solution results in a shift of the binding curve for carbachol to the left without any apparent change of slope, whereas the curve for methylatropinium bromide is unaltered (Taylor & Young, unpublished observations). What structural element is modified by the hypotonic treatment leading to the long-lasting changes in carbachol binding, and in particular whether this is an effect on the receptor macromolecule or its supporting membrane, is unknown. In an attempt to explore the relationship between membrane structure and receptor conformation we have examined the effect of chlorpromazine, a drug whose membrane actions have been extensively studied (Seeman, 1972).

Chlorpromazine, added 30 min prior to the mustard and present throughout, acted as a competitive inhibitor of [^{3}H]-PrBCM binding ($K_a = 1.4 \times 10^6$ M^{-1}), in agreement with Miller & Hiley (1974). However, treatment of the muscle strips for 30 min at room temperature (22°) with higher concentrations of chlorpromazine ($10^{-5} - 10^{-4}$ M), corresponding to the prelytic-lytic range on erythrocytes, resulted in a decrease in [^{3}H]-PrBCM binding measured after 60 min washing in Krebs solution alone. Atropine (10^{-6} M) did not prevent this long-lasting effect of chlorpromazine. The loss of binding was apparently not due to solubilization of receptor material since 10^{-4} M chlorpromazine failed to remove the tritium from strips previously labelled with [^{3}H]-PrBCM. The curve for the inhibition by methylatropinium bromide of [^{3}H]-PrBCM binding to strips pretreated with 2×10^{-5}M chlorpromazine was unaltered from that to normal strips (best fit parameters: Hill slope, 0.99; K_a, 1.2×10^8 M^{-1}; non-specific binding of [^{3}H]-PrBCM, 13%). In contrast the mean Hill slope of the binding curve deduced for carbachol was increased from 0.4 in normal strips to 0.6 in chlorpromazine pretreated strips, with a small increase in the ED_{50}. Thus pretreatment of longitudinal muscle strips with prelytic concentrations of chlorpromazine has an effect on the binding curve for carbachol similar to that of prior desensitization.

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Is the latency of parasympathetic nerve effects due to a slow activation of muscarinic receptors? An iontophoretic study.

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The latency between parasympathetic nerve stimulation and the response of the smooth muscle membrane is in the range 0.07-0.4 s (Gillespie, 1962; M.R. Bennett, 1966; Ohashi & Ohga, 1967; T. Bennett, 1969; Furness, 1969; Ito & Kuriyama, 1971). A possible explanation of this latency is that it is caused by a combination of slow conduction and synaptic delays in the nerve pathways by which excitation reaches the muscle membrane. If this was the case then one would expect the response to iontophoretically applied acetylcholine or carbachol to have a latency much shorter than 0.1 second.

Experiments were therefore done in which carbachol was applied from iontophoretic pipettes (resistance about 200 MΩ) to the surface of smooth muscle from guinea-pig ileum and taenia in isotonic or in sucrose hypertonic physiological salt solution. Simultaneously intracellular records were made from the smooth muscle by microelectrode which was inserted as close as possible to the tip of the carbachol pipette. In most experiments the distance between electrode and pipette was proably not more than 20 μm.

In isotonic solution spikes were often discharged spontaneously. When carbachol was applied iontophoretically no latencies less than about 0.1-0.2 s were observed. The depolarization produced by carbachol invariably triggered a spike or spikes and the electrode was usually dislodged by the contraction of the muscle.

In sucrose hypertonic solution the muscle does not contract and the membrane potential is stable. The sensitivity to carbachol (either applied in the perfusate or iontophoretically) was reduced. Iontophoretic application of carbachol produced small depolarizations of up to 10 mV. Their latency was again never less than 0.1-0.2 seconds.

In these experiments carbachol was released onto the surface of the muscle strip. Presumably therefore it must first cross a layer of serosal cells before it can affect the muscle. By 0.1 s after beginning release of carbachol, it can be calculated that a patch of muscle surface some 100 μm in diameter must be exposed to a suprathreshold concentration of carbachol in these experiments. The observed latency could be explained by invoking some special property of the serosa or associated connective tissue. However, Purves (1974) observed similar latencies when acetylcholine was applied iontophoretically to cultured taenia smooth muscle cells where presumably serosa is absent. A simple explanation of the latencies observed in his experiments and in mine, or following parasympathetic nerve stimulation, is that it represents mainly the time required to produce the responses associated with activation of the muscarinic receptor.

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