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Atropine-resistance of the urinary bladder innervation

The vertebrate urinary bladder is provided with a parasympathetic excitatory innervation. However, while the excitatory effects of acetylcholine on the bladder muscle are strongly antagonized by atropine or hyoscine, the nerve-mediated responses persist with only slight reduction in amplitude (Langley & Anderson, 1895). This evidence has led Henderson & Roepke (1934) and Ambache & Zar (1970) to argue that the excitatory innervation of the bladder is, at least in part, not cholinergic. Other workers have maintained that the innervation is solely cholinergic and have put forward apparently credible theories to explain the inability of muscarinic antagonists to prevent neuromuscular transmission. There are two primary conditions under which muscarinic antagonists such as atropine would not prevent cholinergic transmission to the bladder muscle. First, the receptors specifically occupied by acetylcholine released from nerves could be physically inaccessible to atropine. Second, atropine may reach the receptors but be unable to prevent acetylcholine from occupying the receptors.

There is no evidence to support the suggestion of Carpenter & Rand (1965) that the acetylcholine receptors in the neuromuscular junctions of the bladder are inaccessible to atropine.

Electron microscopic studies have not revealed the existence of any barriers isolating nerve-muscle complexes from the remaining extracellular space (Caesar, Edwards & Ruska, 1957; Thaemert, 1963; Nagasawa & Mito, 1967). In fact the relation between axons and smooth muscle cells in the bladder is similar to the arrangement found in the adrenergically-innervated vas deferens (Merrillees, 1968), yet neuromuscular transmission in the vas deferens is susceptible to blockade by competitive antagonists of α-adrenergic actions (e.g. Boyd, Chang & Rand, 1960). There is therefore no reason to believe that atropine cannot similarly reach all cholinergic receptors in the urinary bladder.

Since atropine is evidently able to penetrate into the neuromuscular junction, its inability to prevent acetylcholine from occupying the receptors indicates that either atropine is displaced from the receptors competitively by high local concentrations of acetylcholine (Huković, Rand & Vanov, 1965) or atropine cannot occupy the cholinergic receptors, i.e. they are not muscarinic. The suggestion that acetylcholine displaces atropine from muscarinic receptors competitively requires that either the amount of acetylcholine released is greater or the width of the synaptic cleft is smaller

in the bladder than in other, atropine-sensitive cholinergic neuromuscular junctions. This theory was tested, using preparations of rat and guinea-pig urinary bladders, bisected in the sagittal plane and suspended in McEwen solution (McEwen, 1956) at 35°. The nerves in the bladder wall were stimulated at 1–6 Hz with pulses of 0·2–1 ms for periods of 10 s via platinum ring electrodes placed around the preparations. The excitatory responses to electrical stimulation were abolished by tetrodotoxin (5×10^{-7} g/ml), showing that there was no direct stimulation of the muscle.

The effects of hyoscine were tested on responses to nerve stimulation and to acetylcholine in preparations treated with pentolinium (5×10^{-5} g/ml) to prevent nicotinic actions of acetylcholine. Hyoscine (3×10^{-7} g/ml) reduced nerve-mediated responses by from 17 to 41%. However, the concentration of hyoscine could be raised to 10^{-3} g/ml without reducing the responses to nerve stimulation by more than 43% (Fig. 1); single stimuli were still effective in causing contractions. Hyoscine (10^{-3} g/ml) abolished responses to acetylcholine at 5×10^{-3} g/ml, and, even at 10^{-2} g/ml, acetylcholine caused only a slight contraction. If the transmitter substance released by the excitatory nerves is acetylcholine, to be consistent with these results the concentration of acetylcholine reaching the receptors would have to exceed 5×10^{-3} g/ml.

The concentration of acetylcholine reaching the receptors could be estimated if both the total amount released by stimulation and the "synaptic volume" into which it is released were known. Two studies have been made of the acetylcholine output from stimulated bladders (Carpenter & Rand, 1965; Chesher, 1967). The highest output per pulse reported, for rat bladders stimulated at 1 Hz, is $6{\cdot}3 \times 10^{-10}$ g per g tissue (Carpenter & Rand, 1965), assuming a bladder weight of 100 mg (Chesher, 1967). For comparison, the output from guinea-pig ileum per pulse during stimulation at 1 Hz is 8×10^{-10} g per g tissue (Paton & Zar, 1968), indicating that acetylcholine is

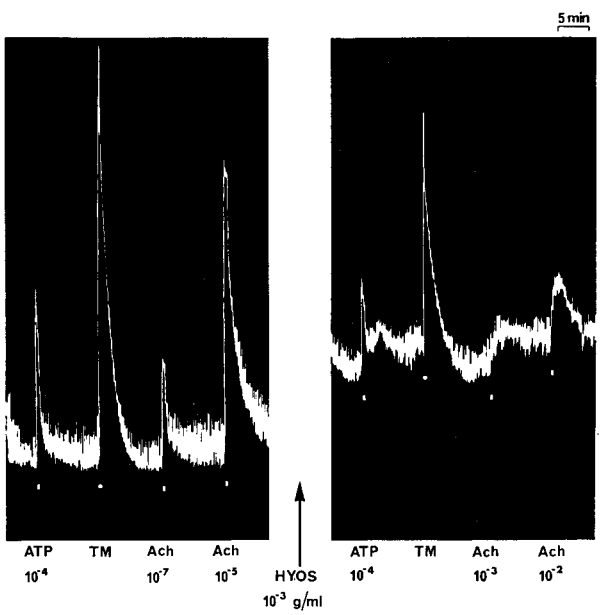

FIG. 1. The effect of a high concentration of hyoscine on excitatory responses of the isolated guinea-pig bladder. Preparation treated with pentolinium, (5×10^{-5} g/ml). The first panel shows contractions produced by adenosine triphosphate (ATP, 10^{-4} g/ml for 30s), transmural stimulation (TM) at 5 Hz for 10 s and acetylcholine (Ach, 10^{-7} and 10^{-5} g/ml for 30 s). In the second panel, 90 min after the addition of hyoscine (HYOS, 10^{-3} g/ml), contractions produced by adenosine triphosphate and transmural stimulation are present, though reduced. Note that acetylcholine (10^{-3} g/ml) is without effect, whereas 10^{-2} g/ml causes contractions. Time marker, 5 min.

released from bladder tissue in normal quantities. It is probably released, as proposed for adrenergic autonomic nerves (Malmfors, 1965), from the varicosities of the terminal axons. To achieve the most favourable conditions for displacement of atropine to occur, this quantity of acetylcholine must be released into the smallest possible synaptic volume. A minimal estimate of synaptic volume can be arrived at as follows.

1. The closest approximation of axon varicosities to bladder muscle cells is 20 nm (Caesar & others, 1957; Thaemert, 1963). Varicosity diameter is about 1μm. The 'unit synaptic volume', defined as the discoid space between a varicosity and a closely apposed muscle cell is therefore no less than 0·016 μm^3.

2. As a least estimate, there is one 20 nm neuromuscular apposition per muscle cell in the bladder (Caesar & others, 1957; Nagasawa & Mito, 1967). The least number of muscle cells in a gram of tissue can be calculated, from the greatest measured volume of single cells (3500 μm^3 in guinea-pig vas deferens) (Merrillees, 1968) and the smallest measured intracellular space of smooth muscle tissues (about 60% of the tissue volume) (Burnstock, 1970), to be $1{\cdot}7 \times 10^8$ cells/g (assuming a specific gravity of 1). The least total number of 'unit synaptic volumes' is $1{\cdot}7 \times 10^8$ per g tissue.

3. From 1 and 2, the least estimate of 'total synaptic volume' is $2{\cdot}7 \times 10^6$ μm^3/g tissue.

To achieve a maximal estimate of the concentration of acetylcholine reached at the muscle cells, it is assumed that the total measured output is released into the 'synaptic volume' as defined above, i.e. there is no release from varicosities further than 20 nm from a muscle cell. If this is assumed, the concentration of acetylcholine at the muscle cells is $2{\cdot}3 \times 10^{-4}$ g/ml. It can be seen that this concentration is at least 20-fold smaller than the least concentration shown above to excite the bladder in the presence of hyoscine (10^{-3} g/ml). If more realistic assumptions, especially about the mode of release of acetylcholine, are made, the concentration achieved will be even smaller. It seems clear that the atropine-resistance of the excitatory nerves cannot be explained in terms of displacement of atropine from receptors by acetylcholine.

The alternative explanation for the inability of atropine to prevent occupation of receptors by acetylcholine is that the receptors are not muscarinic. Whether these receptors are postulated to be nicotinic or of any other cholinergic type, the fact remains, as shown above, that acetylcholine would have to reach the bladder muscle in a concentration greater than 5×10^{-3} g/ml, a concentration which cannot be achieved physiologically. Clearly this theory does not explain the atropine-resistant transmission.

The partial blockade of nerve-mediated responses by low concentrations of muscarinic antagonists (Ursillo, 1961) and the enhancement of responses by anticholinesterase drugs (Edge, 1955) show that part of the bladder innervation is cholinergic. The only direct evidence that the innervation is wholly cholinergic is the observation that hemicholinium-3 and botulinus type D toxin severely reduce excitatory transmission to the bladder (Huković & others, 1965; Carpenter, 1967). While this blockade might be due to the action of these agents in preventing acetylcholine release, both are known to have additional actions, at least against adrenergic transmission (Chang & Rand, 1960; Rand & Whaler, 1965). The blockade produced by both agents, although consistent with the theory that the nerves are entirely cholinergic, clearly does not prove the case. However, since from the arguments here advanced the theories explaining atropine-resistance in terms of cholinergic transmission would appear not to be feasible, the opinion of Henderson & Roepke (1934) that the innervation also contains a non-cholinergic excitatory component would seem to hold.

If there is a non-cholinergic excitatory innervation of the bladder, it must be asked what transmitter substance is released. Evidence has already beeen presented against transmitter actions of catecholamines, 5-hydroxytryptamine, bradykinin, and histamine (Huković & others, 1965; Edvardson, 1968; Gyermek, 1962; Ambache & Zar, 1970). Another possibility is that the transmitter substance is adenosine triphosphate (ATP), which causes contraction of canine bladder muscle (Matsumura, Taira & Hashimoto, 1968) and which has been suggested as a transmitter substance released by autonomic nerves in the gut (Burnstock, Campbell & others, 1971) and in the portal vein (Hughes & Vane, 1967). A strong contraction of the guinea-pig bladder was obtained with ATP (10^{-4} g/ml) in the present experiments (Fig.1). The contraction was not affected by tetrodotoxin (5×10^{-7} g/ml) and therefore appears to be due to a direct action on the muscle. Hyoscine (3×10^{-7} g/ml) did not reduce the ATP response; even at 10^{3} g/ml, hyoscine did not prevent ATP contractions (Fig. 1). It can be seen that this experiment provides better evidence, however scant, for ATP than for acetylcholine as the transmitter substance released by the atropine-resistant nerves.

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