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Presence of non-cholinergic motor transmission in human isolated bladder

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Abstract—Atropine-sensitivity of the motor transmission in the isolated detrusor preparation from human bladder has been examined. The preparations were contracted by electrical field stimulation consisting either of short trains of pulses or of long trains of pulses. Part of the stable response to short-train stimuli (28%) was resistant to atropine, was not potentiated by physostigmine and was blocked by tetrodotoxin. The stable responses to long-train stimuli were fully blocked by atropine. It is concluded that the detrusor of the bladder in man, in common with other mammalian species, contains a non-cholinergic component in its motor transmission, and that prolonged stimulation with long-train stimuli causes an extinction of the non-cholinergic motor transmission, probably through depletion of transmitter stores in the nerve-terminals.

Partial atropine-resistance of motor transmission of isolated bladder preparations is well recognized in many mammalian species (Taira 1972) and is believed to be due to the presence of a non-cholinergic motor neurotransmitter in the autonomic nerve supply to the detrusor (Ambache & Zar 1970). The human bladder seems to be an exception in that most reports indicate a complete abolition of motor transmission by atropine or atropinic agents (Nergardh & Kinn 1983; Sibley 1984; Kinder & Mundy 1985). We have examined whether the reported full atropine-sensitivity of human bladder is the result of a genuine absence of a non-cholinergic element in its motor transmission or is caused by the experimental protocol involving the prolonged electrical stimulation given in most investigations. On the assumption that the stores of the non-cholinergic transmitter in human bladder isolated preparations are small and non-renewable owing to the absence of local non-cholinergic neuronal cell bodies, prolonged electrical field stimulation would cause their depletion. To distinguish between the two possibilities, in the present investigation we have examined the effect of atropine on the transmission under two experimental protocols: one in which the field stimulation was limited to the minimum essential to evoke consistent and reproducible mechanical responses, and the other in which the field stimulation, comprised of long trains of pulses, was delivered at short intervals.

Materials and methods

A total of 48 experiments was performed on strips of bladder obtained from 13 patients during operations for lower urinary tract disorders. Thin strips of the bladder, cut in the longitudinal axis of the detrusor, 1·0–1·5 cm long, 0·15–0·2 cm wide, were prepared and set up in 1 mL organ baths. For recording isometric contractions, the strips were placed between platinum electrodes in Krebs-Henseleit solution containing indomethacin 1 μM (composition mM: NaCl 118, KCl 4·7, $CaCl_2$ 2·5, $MgSO_4$ 1·2, $NaHCO_3$ 25, KH_2PO_4 1·2, glucose 11), at 37°C, bubbled with 5% CO_2 in oxygen. A preliminary 90 min equilibration period was allowed before proceeding with experimentation. At least one bladder strip from each patient was reserved for each of the following two experimental procedures. *Protocol 1* (short trains at long intervals): following the preliminary equilibration period, the preparation was subjected to electrical field stimulation consisting of trains of 10 pulses at 10 Hz, each pulse of 0·1 ms duration and of supramaximal voltage, the train-frequency being one every 100 s. When the contractile responses to the train of pulses became reproducible the preparation was exposed to atropine for the remainder of the experiment. Atropine 0·5, 1, and 3 μM was used in four preliminary experiments to establish that maximal inhibition of the electrically-evoked contractions was obtained at 0·5–1 μM. Atropine, 3 μM, therefore, was routinely used in the main experiments. After obtaining stable responses to electrical field stimulation (usually after 15–20 min exposure to atropine), the preparation was treated with physostigmine 1 μM; 15 min later the neurogenicity of the electrically-evoked contraction was checked by testing its sensitivity to tetrodotoxin 0·5 μM. *Protocol 2* (long trains at short intervals): At the end of the equilibration period, the preparation was contracted at 30 s intervals with trains of 90 pulses; other parameters of stimulation were identical to those in Protocol 1. The responses were allowed to stabilize before exposing the preparation to atropine 3 μM.

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Results and discussion

In the Protocol 1 experiments (short trains at long intervals),

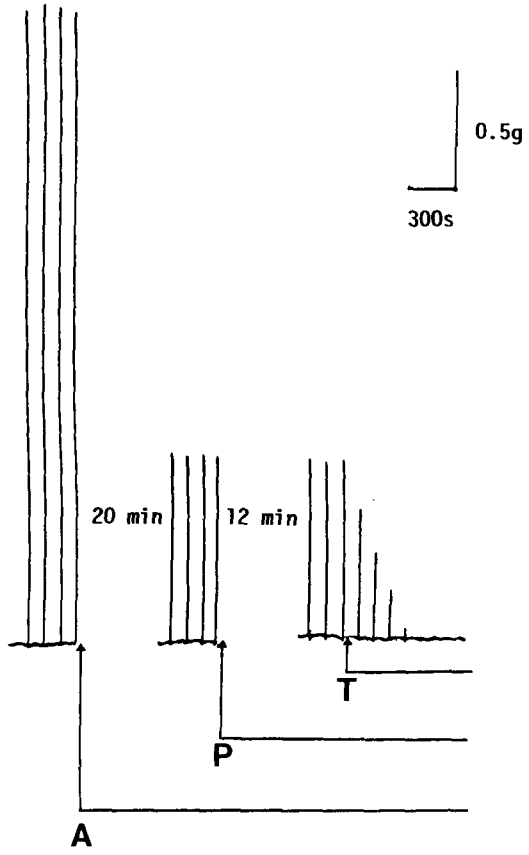

FIG. 1. Contractions of the human detrusor in response to electrical field stimulation (Protocol 1: 10 pulses every 100 s): The effect of atropine, 3 μM, followed by physostigmine 1 μM and tetrodotoxin 0·5 μM. The arrows mark the introduction of atropine (A), physostigmine (P) and tetrodotoxin (T). The first contractile responses of the middle and last panels were recorded 20 and 12 min after injection of atropine and physostigmine, respectively.

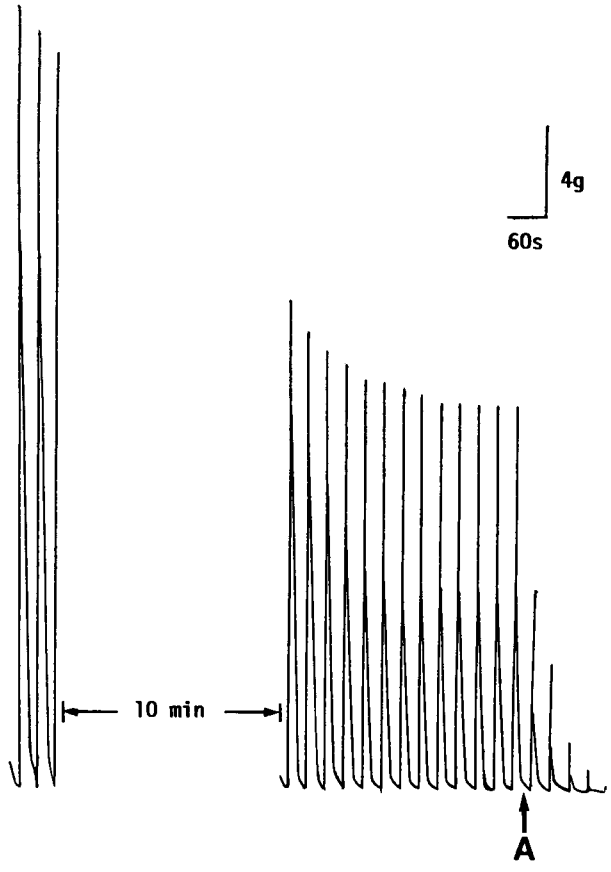

FIG. 2. Contractions of the human detrusor in response to electrical field stimulation (Protocol 2: 90 pulses every 30 s): Atropine 3 μM was introduced at the arrow marked A, 18 min after the start of the repetitive electrical stimuli. Note the rapid and complete blockade of motor transmission by atropine.

atropine, 3 μM, caused some inhibition of the electrically-evoked response in all preparations (e.g. see Fig. 1). The degree of maximal inhibition varied in different preparations, but was never total (mean $\pm$ s.e.m. % inhibition = $72 \pm 3{\cdot}5$). Exposure to the anticholinesterase, physostigmine 1 μM, did not produce an augmentation of the atropine-resistant contraction (Fig. 1), further discounting the possibility that the contraction in the presence of atropine, despite its atropine-insensitivity, was still somehow cholinergic. The electrically-evoked atropine-resistant contraction was readily and completely abolished by tetrodotoxin, 0·5 μM (Fig. 1), thus signalling its neurogenic origin. In Protocol 2 experiments (long trains of pulses at short intervals), administration of atropine fully blocked the electrically-evoked responses and the extinction of transmission was achieved after less than 5 min exposure to atropine (Fig. 2). These experiments therefore suggest that stable responses of this tissue to repetitive long-train stimuli are entirely cholinergic in contrast to the responses to short-train stimuli which contain a substantial non-cholinergic component (about 30%). Since the main difference between the two experimental protocols lies in the total number of pulses delivered to the preparations before their exposure to atropine, it seems reasonable to suggest that the absence of a non-cholinergic motor transmission to Protocol 2 experiments reflects a depleting effect of repetitive long-train stimuli on the intra-neuronal non-cholinergic motor transmitter stores.

It is of considerable relevance to estimate the total number of pulses delivered to human isolated detrusor preparations in experiments in which other workers, for example Kinder & Mundy (1985) and Sibley (1984), achieved total abolition of motor transmission with atropine like drugs. It is not possible to be precise on this, nevertheless certain broad conclusions can be drawn from the descriptions of their experiments. In the experiments of Kinder & Mundy a minimum of 3690 pulses were delivered to each preparation before exposure to atropine, while in Sibley's investigation, the minimum number of pulses delivered before atropinization was in excess of 775. In our Protocol 1 experiments the responses to repetitive stimuli stabilized within 10–15 min and therefore the total number of pulses delivered before exposure to atropine was well below 100. It follows from this that the experiments of Kinder & Mundy and of Sibley, matched our Protocol 2 experiments (e.g. about 4000 pulses in Fig. 2.) more closely than our Protocol 1 experiments in the total number of pulses delivered before atropinization, and it is not without significance that atropine blocked fully the contractile responses to electrical stimulation in all but the Protocol 1 experiments.

The results therefore clearly demonstrate the presence of a non-cholinergic component in the motor transmission of isolated human bladder preparations and in this respect no radical qualitative difference exists between man and other mammalian species.

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