The effect of imipramine on isolated innervated guinea-pig and rat urinary bladder preparations

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Imipramine and amitriptyline are the main drugs currently employed in the treatment of functional enuresis. Their mechanism of action is ill-understood, and one of the likely mechanisms suggested is that they act through their peripheral anticholinergic effect (see Kolvin, 1975 for references). However, studies carried out by Diokno, Hyndman & others (1972) on patients with uninhibited neurogenic bladders failed to demonstrate any parasympatholytic response with imipramine while propantheline could completely abolish bladder contractions. The same authors found imipramine to be superior to propantheline in the control of functional enuresis. These findings militate against the assumption that the effectiveness of imipramine in functional enuresis is due to its peripheral atropine-like action. In this context it may be mentioned that imipramine is only 1/157 as potent as atropine (Sigg, 1959). We, therefore, studied the effect of imipramine on guinea-pig and rat isolated innervated urinary bladder preparations. These cholinergic preparations are known to be atropine resistant and the maximum neuronal blockade produced by atropine or hyoscine is only mild (Huković, Rand & Vanov, 1965; Weetman, 1972).

Isolated innervated guinea-pig (Weetman, 1972) and rat (Huković & others, 1965) urinary bladder preparations were set up in 30 ml organ baths containing Tyrode solution, bubbled with 5% CO_2 in oxygen, maintained at 33–35°. Isotonic contractions were recorded on a moving kymograph using a frontal writing lever (magnification × 7). The preparations were neuronally stimulated by rectangular pulses (10–15 V, 0·5 ms duration) at 10 Hz for 5 s every 2 min; in three experiments high (50 Hz) and low (2 Hz) frequencies were also employed. The effect of imipramine alone, and that in presence of atropine was observed on neuronally evoked bladder responses.

To compare the atropine blockade with that produced by a nearly equi-anticholinergic dose of imipramine five paired experiments were set up using the isolated innervated guinea-pig hemi-bladder preparation (Dhattiwala & Dave, 1975). This preparation gives consistent responses comparable to those obtained with the whole bladder preparation. In each pair the maximum blockade of neuronally evoked responses produced by atropine (0·1 μg ml^{-1}) on one hemi-bladder and that produced by imipramine (10 ng ml^{-1}) on the counter half of the bladder was separately calculated. The data were pooled and the difference in the mean block between the two groups was statistically analysed using Student's *t*-test.

The following drugs were used: imipramine hydrochloride (Tofranil), atropine sulphate and acetylcholine chloride; doses refer to salts.

Imipramine (25 μg ml^{-1}) blocked the neuronally evoked responses of the guinea-pig bladder by 79% ± 3 (mean ± s.e., n = 14) of the control: responses to added acetylcholine were completely blocked. The blockade was quick in onset and rapidly progressed to the maximum in 20–30 min. Recovery of neuronal responses following the blockade was only partial (<50%) after 1 h of repeated washes; recovery of acetylcholine responses was quick and was almost complete in 1–1½ h. At the time of full recovery of acetylcholine responses the partial blockade of the neuronal responses still persisted (Fig. 1). In three experiments, 50 μg ml^{-1} of imipramine completely blocked the neuronally evoked responses.

Imipramine (25 μg ml^{-1}, n = 3) blocked the responses at 2 Hz and 50 Hz to the same extent as it blocked the responses at 10 Hz.

In three experiments carried out in the presence of atropine (5 μg ml^{-1}), neuronally induced responses of guinea-pig bladder were blocked 70–85% by imipramine (25 μg ml^{-1}).

Neuronally evoked responses of the rat bladder were inhibited by imipramine (20 μg ml^{-1}) by 86% ± 4 (mean s.e., n = 10) of the control; in two of these experiments the blockade was complete. Recovery of acetylcholine responses were completely blocked. Recovery of acetylcholine responses was more than 80% in 1 h but recovery of the neuronal responses was very slow and only partial (<30%) at the end of 1 h of repeated washing.

Spontaneous activity of the guinea-pig and the rat bladders was markedly increased in some experiments at the time of imipramine-induced blockade.

In the paired experiments atropine (0·1 μg ml^{-1}) blocked the neuronally evoked responses by 13% ± 4 (mean ± s.e.) while imipramine (10 μg ml^{-1}) blocked the responses by 38% ± 4 (mean ± s.e.)—the difference was significant ($P<0.01$).

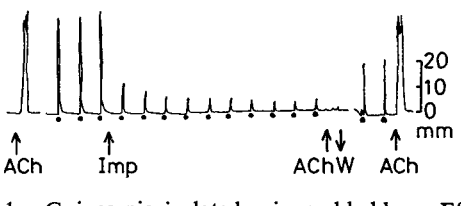

FIG. 1. Guinea-pig isolated urinary bladder. Effect of imipramine (Imp, 25 μg ml^{-1}) on neuronal responses (at dots, rectangular pulses, 10 V, 0.5 ms, 10 Hz for 5 s every 2 min), and on acetylcholine (ACh, 0.1 μg ml^{-1}) responses. W, wash. Last panel shows recovery 1 h after wash.

The results have shown that the imipramine-induced marked blockade of the neuronally evoked responses of the guinea-pig and rat bladders—the preparations known to be atropine resistant—could not be due to its atropine-like action. Imipramine exhibits local anaesthetic action (Sigg, 1959; Ritchie & Greengard, 1961) and procaine has been reported to block the neuronally evoked responses of the guinea-pig (Weetman, 1972) and the rat bladders (Huković & others, 1965; Dhattiwala, Jindal & Kelkar, 1970). It therefore seems reasonable to attribute the powerful blocking effect of imipramine to its procaine-like action at the nerve terminals and the adjacent effector cell membrane; only the latter recovers fully on repeated washes as shown by full recovery of acetylcholine responses and only partial recovery of the neuronal responses. To what extent the local anaesthetic action of imipramine might be involved in human nerve-bladder transmission is a problem worth investigating.

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The effect of methysergide on 5-hydroxytryptamine turnover in whole brain

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Considerable evidence has been accumulated to support the concept that methysergide bimaleate acts by blocking 5-hydroxytryptamine (5-HT) receptors (Nerebski, Romanowski & Kadjiela, 1962; Dewhurst & Marley, 1965; Koella, 1966; Banna & Anderson, 1968; Clineschmidt & Anderson, 1970; Marin, 1970). We have undertaken to determine the effect of methysergide on concentrations of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in whole brain and to consider the results in terms of the hypothesis that 5-HT receptor blockade causes increased 5-HT turnover. To examine the effect of simultaneous dopaminergic blockage relevant to the action of methiothepin, experiments were also performed in which haloperidol was given simultaneously with methysergide.

White male guinea-pigs (225–250 g) were given different dosages of methysergide (UML-491, Sandoz, Inc.) and haloperidol (Haldol, McNeil Labs., Inc.) subcutaneously. Animals were housed under conditions of standard laboratory lighting and had free access to food and water. Upon completion of desired treatment intervals animals were decapitated, brains rapidly removed and plunged into liquid nitrogen. 5-HT and 5-HIAA were determined spectrophotofluorometrically by the method of Curzon & Green (1970).

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Table 1 shows the effect of various drug regimes on whole brain 5-HT and 5-HIAA concentrations (expressed as a percentage of controls). Methysergide (3 mg kg^{-1}) significantly decreased the 5-HT content of whole brain at 15 min ($P < 0.05$). At 60 to 120 min, there was no significant alteration in whole brain 5-HT with methysergide (3 and 10 mg kg^{-1}). Haloperidol 0·5 mg kg^{-1}, failed to alter 5-HT concentrations at 120 min while 10 mg kg^{-1} caused a significant increase in whole brain 5-HT at 120 min ($P < 0.01$). The simultaneous administration of methysergide and haloperidol at two different dosages had no effect on whole brain 5-HT at 120 min. Various drug regimes were ineffective in altering whole brain 5-HIAA concentrations (all > 0.2).

The failure of methysergide to alter 5-HIAA concentrations in the present study suggests that it does not change 5-HT turnover in whole brain. Andén, Corrodi & others (1968) also noticed no increase in 5-HT synthesis after methysergide (0·2 mg kg^{-1}, i.p.) in conjunction with a 5-HT synthesis inhibitor. These findings raise questions concerning the site(s) of action of methysergide and the generality of synaptic regulatory mechanisms drawn from the study of other neurotransmitter systems.

The increase in homovanillic acid (HVA) concentrations following receptor blockade is probably a consequence of increased dopamine turnover (Andén,