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Analysis of the Anticholinergic and Musculotropic Effects of Desmethylimipramine on the Rabbit Urinary Bladder

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Summary. The anticholinergic and musculotropic relaxant properties of desmethylimipramine (DMI) on smooth muscle were investigated utilizing cholinergic radioligand receptor binding and in-vitro muscle bath techniques. Receptor binding studies revealed the direct antimuscarinic potency of DMI to be 1/480th that of atropine. Muscle bath studies characterized discrete antimuscarinic and musculotropic actions. An initial, competitive antimuscarinic action could be separated from a delayed onset, noncompetitive musculotropic action by altering the time between the addition of DMI and the addition of bethanechol to the smooth muscle bath.

Key words: Desmethylimipramine, Musculotropic, Anticholinergic, Cholinergic receptor binding.

Introduction

The mechanism of action of many drugs which are used clinically to alter voiding function is not well understood. Tricyclic antidepressant drugs, particularly imipramine, have come to be accepted for the treatment of enuresis and a number of other disorders of micturition [3–5, 10–15]. Empirically, this agent seems to increase outlet resistance in the area of the bladder base and proximal urethra and to decrease bladder contractility [15]. Clinical and experimental studies have implicated both anticholinergic and direct musculotropic actions as being responsible for this latter effect.

The purpose of this study was to better define and characterize the relative contributions of the antimuscarinic and musculotropic relaxant mechanisms to the overall action of desmethylimipramine on the smooth muscle of the urinary bladder.

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Methods

Muscle Bath Studies

The urinary bladders of 18 male New Zealand White rabbits were removed under ketamine-xylazine anesthesia. The bladder was rapidly dissected free of fat, and the body and base were separated at the level of the ureteral orifices. The bladder body was divided into four longitudinal strips of equal size, which were placed in individual muscle baths containing 30 ml of Tyrode's solution containing glucose (1 mg/ml) and equilibrated with a gas mixture of 95% O_2 and 5% CO_2. After 20 min of equilibration, 1 gm of tension was placed on each strip, and the strips were maintained in the oxygenated buffer for an additional 20 min.

At the end of the equilibrium period, the strips were exposed to a specific sequence of pharmacologic agents.

Receptor Binding Studies

The urinary bladders of eight male New Zealand White rabbits were rapidly removed under pentobarbital anesthesia. The bladders were dissected free of serosa and mucosa, divided between bladder base and body at the level of the ureteral orifices, and frozen in approximately 1-gm sections in liquid nitrogen until testing.

The frozen tissue samples were rapidly weighed and homogenized (50 mg/ml) in ice-cold phosphate buffer (50 mM), pH 7.4, using a Brinkman Polytron homogenizer. Only bladder body specimens were utilized. The homogenate was passed through a double layer of gauze and used immediately for the receptor binding assay.

The relative ability of DMI (or atropine) to compete for the muscarinic receptor was determined as follows. 200 µl of tissue homogenate (50 mg/ml) were incubated in the presence of 10 nM ^{3}H-quinuclidinyl-bezilate (^{3}H-QNB)[1] and various concentrations of the drug under study in a total volume of 280 µl for 30 min at 25 °C. The reaction was stopped by the addition of 4 ml ice-cold buffer (50 mM phosphate, pH 7.4) and the solution was rapidly filtered through a Whatman GFC glass fiber filter. The filter was washed with three 5-ml portions of ice-cold buffer. The filters were then placed in scintillation vials with 5 ml of scintillation fluid, and the radioactivity was determined by liquid scintillation spectrometry.

1 ^{3}H-QNB (30.2 Ci/mMol) was obtained from New England Nuclear. Other reagents were obtained from general commercial sources

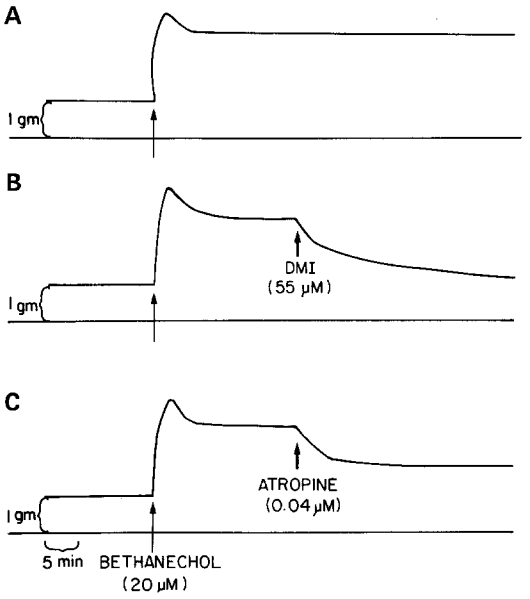


Fig. 1A–C. Effect of DMI on bethanechol-stimulated contraction. Representative tracing of the immediate effects of DMI on submaximal contractile stimulation by bethanechol. The effect of atropine is presented for comparative purposes

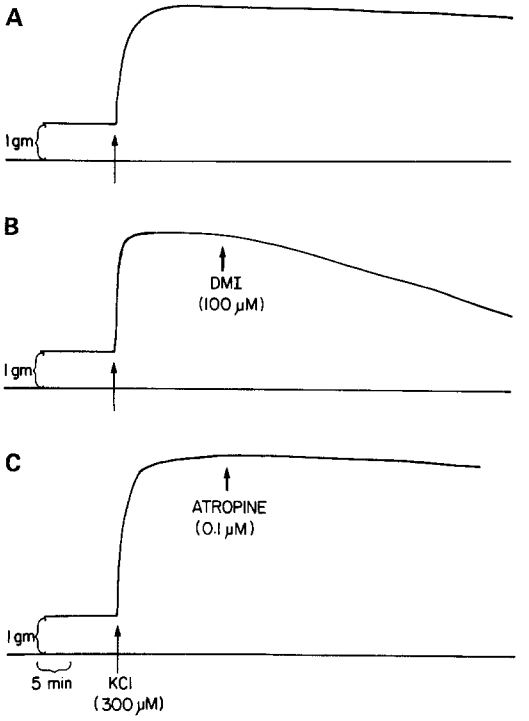


Fig. 2A–C. Effect of DMI on KCl-stimulated contraction. Representative tracing of the immediate effects of DMI and atropine on maximal contractile stimulation by KCl

The amount of ^{3}H-QNB bound in the absence of any drug is equal to the total binding, the amount bound in the presence of 10 μM atropine is equal to the non-specific binding (less than 20% of total binding), and the specific binding is equal to the total binding minus the non-specific binding. The specific binding in the presence of DMI was calculated by subtracting the nonspecific binding from the amount bound in the presence of DMI. The effect of DMI on the specific binding was determined by constructing a dose-response curve and calculating the I_{50}, i.e., the concentration of DMI required to displace 50% of the specific binding.

Results

The Effect of DMI on Bethanechol-Stimulated Contraction

Figure 1 presents a representative tracing of the effects of DMI on bethanechol-stimulated contraction. The effect of atropine has been included for comparative purposes (spontaneous contractions have been eliminated for clarity).

Submaximal contractile stimulation by bethanechol (Fig. 1A) is characterized by an immediate increase in tension followed by a prolonged plateau phase. DMI addition during the plateau phase (Fig. 1B) produced an immediate decrease in tension followed by a continuous decline observed after DMI. Atropine produced an immediate decrease in tension to a stable reduced level.

In contrast, the effect of DMI (2B) and atropine (2C) on KCl-stimulated contraction demonstrates that whereas DMI produced a gradual decline of tension (without the immediate effect observed against bethanechol), atropine had no effect on KCl-stimulated contraction.

Figure 3 presents the dose-response effect of DMI on bethanechol-stimulated contraction. As previously described, DMI was added during the plateau-phase and the effect determined at 5 min (initial effect) and at 30 min (delayed effect). As can be observed, the delayed effect (I_{50} = 9 μM) is significantly more potent than the initial effect (I_{50} = 50 μM). Because of the delayed effect of DMI, each strip was utilized for only one experiment (one concentration of DMI).

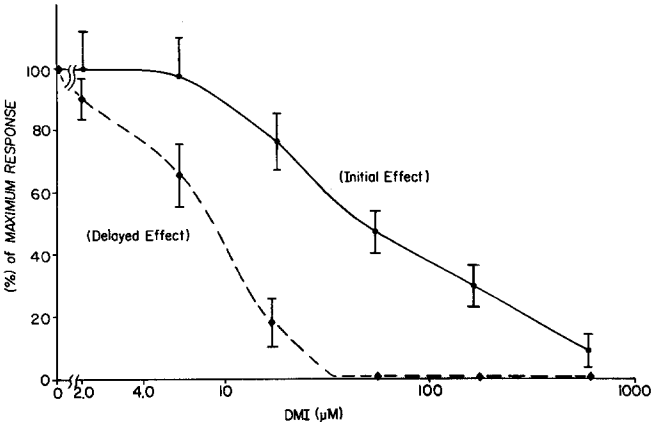


Fig. 3. Dose-response of the initial and delayed effects of DMI on bethanechol-induced contraction. Each point represents the mean ± SE of six to eight separate experiments

The initial effect of DMI could be reversed by the addition of bethanechol within 4 min of the DMI addition (Fig. 4A), whereas the effect at 30 min was not reversible (Fig. 4B). The effect of atropine could be reversed at either time (Fig. 4C).

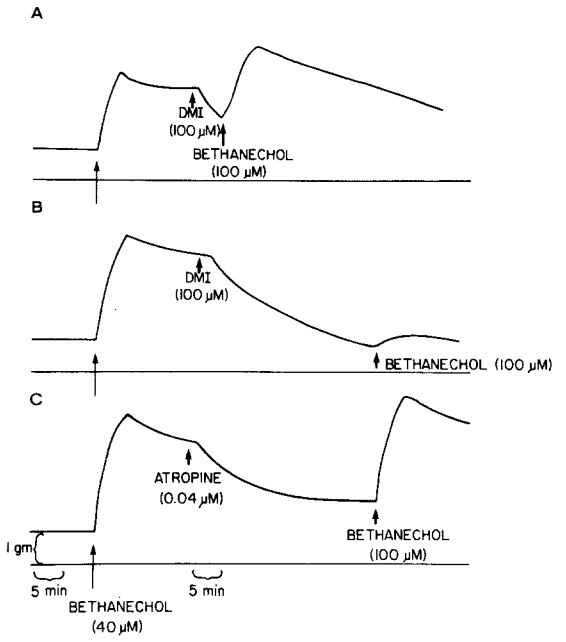


Fig. 4. Reversal of DMI inhibition by bethanechol. Representative tracing of the ability of bethanechol to reverse DMI (and atropine) inhibition

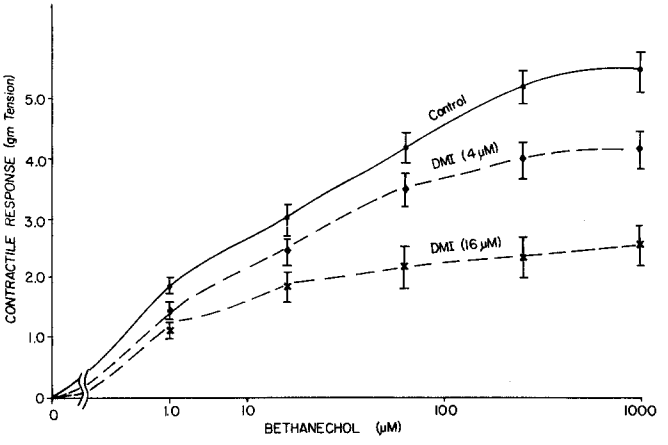


Fig. 5. Noncompetitive inhibition of bethanechol-induced contractions by preincubation with DMI. Each point is the mean ± SE of six to eight separate experiments

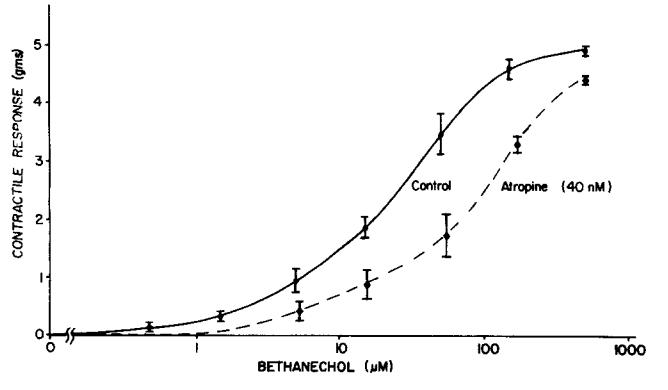


Fig. 6. Competitive inhibition of bethanechol-induced contractions by atropine. Each point is the mean ± SE of six to eight separate experiments

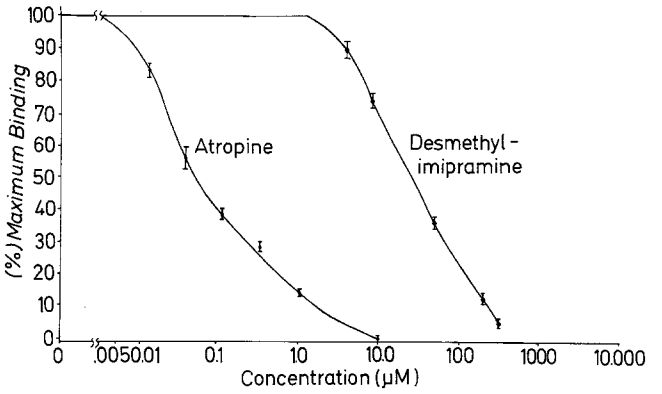
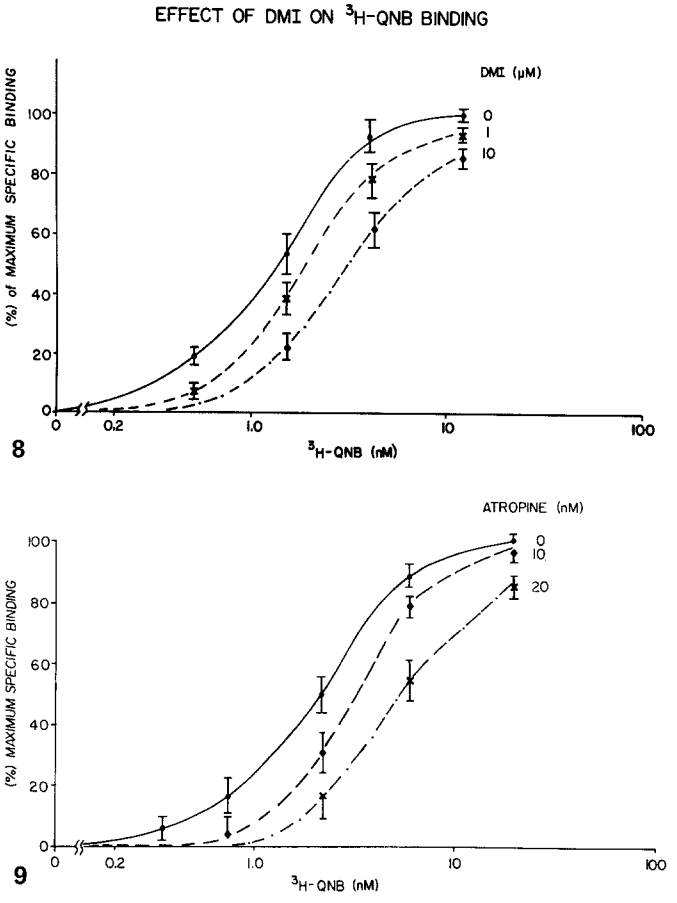


Fig. 7. Displacement of ^{3}H-QNB by atropine and DMI. Each point is the mean ± SE of six to eight separate experiments



Figs. 8 and 9. Competition of DMI and atropine for the muscarinic receptor. Each point is the mean ± SE of six to eight separate experiments

The non-competitive nature of the delayed DMI inhibition is demonstrated in Fig. 5. Muscle strips have been preincubated in the presence of DMI for 20 min prior to performing a dose-response curve to bethanechol. The curves obtained are typical of non-competitive inhibition. In contrast, preincubation with atropine (Fig. 6) demonstrates a competitive inhibition of bethanechol-stimulated contraction.

The Effect of DMI on ^{3}H-QNB Binding

The relative effect of DMI and atropine on the binding of ^{3}H-QNB to muscarinic receptors is presented in Fig. 7. The I_{50} for atropine was 50 nM whereas the 1_{50} for DMI was 24 μM. The competitive nature of both DMI and atropine on ^{3}H-QNB binding is presented in Figs. 8 and 9.

Discussion

Imipramine and other tricyclic antidepressant drugs are of proven efficacy in the therapy of enuresis and of urinary storage disorders secondary to decreased outlet resistance and/or detrusor hyperreflexia and hypertonicity [3–5, 10–15]. Interest in its mechanisms of action has prompted numerous clinical and laboratory investigations [2, 5–9, 16].

Clinical observation and laboratory studies on whole animal preparations and in vitro tissue studies have suggested a myriad of possible mechanisms, including: local anesthetic [4, 13], musculotropic-smooth muscle relaxant [15], inhibition of noradrenaline re-uptake [1, 2], alpha-adrenergic blockade [11], beta stimulation [16], and anticholinergic [9].

The multiple mechanisms of action attributed by various investigators to imipramine prompted this study to 1) isolate and quantify the anticholinergic activity of DMI through competitive radioligand techniques, and 2) separate and identify the antimuscarinic and musculotropic activity of DMI through a detailed in-vitro study of the relationship between bethanechol stimulation and DMI inhibition.

Our results can be summarized as follows:

A. Characteristics of Atropine Response

1) Initial rapid relaxation of a bethanechol-induced contraction (Fig. 1C); 2) Reversibility by bethanechol (Fig. 4C); 3) Competitive inhibition of a bethanechol-induced contraction (Fig. 6); and 4) Failure to inhibit potassium chloride-induced contraction. This is consistent with a competitive muscarinic effect, observed when tested against cholinergic stimulation in vivo.

B. Characteristics of DMI Response

The characteristics of DMI responses can be divided into "initial" competitive (5 min) and "delayed" noncompetitive (30 min) effects. Initial effects were characterized by: 1) Rapid relaxation of the bethanechol-induced response (Fig. 1B); 2) Reversibility by bethanechol (Fig. 4A). This is consistent with an atropine-like antimuscarinic effect which is more rapid in onset than the delayed musculotropic effects.

Delayed effects were characterized by: 1) The continued relaxation of the bethanechol-induced contraction (Fig. 1B); 2) Not reversible by bethanechol (Fig. 4B); 3) The noncompetitive inhibition of bethanechol (Fig. 5); and, 4) The ability to inhibit KCl-induced contraction. These "delayed" effects are consistent with an antispasmodic musculotropic relaxant mechanism of action.

The anticholinergic binding studies demonstrate that while both DMI and atropine are competitive inhibitors at the muscarinic receptor, atropine is nearly 500 times more potent. Thus, DMI has a relatively weak antimuscarinic effect when compared to its more potent musculotropic relaxant action on detrusor contractility.

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