THE EFFECT OF IMIPRAMINE ON ISOLATED SKELETAL MUSCLE PREPARATIONS

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Summary : Imipramine (2-10 $\mu g/ml$) noncompetitively inhibited acetylcholine responses of the frog rectus abdominis muscle, and markedly inhibited the contracture produced by carbachol and succinylcholine without affecting the contracture produced by KCl, caffeine, and chlorpromazine. The twitch responses to indirect and direct stimulation of the rat phrenic nerve-diaphragm and the frog sciatic nerve-gastrocnemius were first augmented and then depressed markedly and irreversibly by imipramine (5-20 $\mu g/ml$). The indirect stimulation was inhibited earlier and to a greater degree than the direct stimulation. The blockade in the nerve-sartorius developed and progressed quickly without prior augmented responses, and with a parallel time course for indirect and direct stimulation.

On the frog rectus, physostigmine antagonised whereas d-tubocurarine and $CaCl_2$ increased the imipramine-induced inhibition. In the nerve-muscle preparations, physostigmine, $CaCl_2$ and KCl did not affect the neuromuscular blockade produced by imipramine; tubocurarine (0.05 $\mu g/ml$) markedly increased the blocking effect of imipramine (20 $\mu g/ml$) on the rat phrenic nerve-diaphragm. The results have been discussed in relation to the memberane stabilizing and the calcium releasing actions of imipramine.

Key words : Imipramine neuro-muscular blockade

INTRODUCTION

Imipramine has been shown to block powerfully the nerve-muscle transmission in isolated urinary bladder preparations where the blockade was postulated to be due to the local anaesthetic action of imipramine exerted pre and postjunctionally (6). Antiacetylcholine effects of imipramine-like drugs on skeletal muscles have been reported (2,11). Using intracellular recording techniques, Chang and Chuang (5) have presented a comprehensive analysis of the neuromuscular blocking action of desipramine and imipramine on the rat phrenic nerve-diaphragm and have discussed their membrane stabilizing property in relation to that of procaine. This paper reports the neuromuscular blocking effect of imipramine on different skeletal muscle preparations in relation to its membrane stabilizing action.

MATERIALS AND METHODS

Rectus abdominis muscle of frog (Rana pipiens) :

The method described by Burn (4) was followed using a 25 *ml* organ bath filled with frog-Ringer's solution (25°-27°C), bubbled with O_2.

Phrenic nerve-diaphragm preparation of rat :

The preparation was set up according to the method of Bulbring (3). The muscle was suspended in 50 *ml* organ bath filled with double dextrose-Tyrode solution (30° - 32°C), bubbled with O_2.

Sciatic-gastrocnemius preparation of frog :

The frog was pithed and the skin over the leg was removed. The sciatic nerve was isolated from surrounding tissue to the point of entry into the gastrocnemius muscle. The nerve and the gastrocnemius muscle were then removed from the frog. The muscle was mounted in a 50 *ml* organ bath filled with frog-Ringer's solution (25°-27°C) bubbled with O_2, and contractions to nerve or muscle stimulation were recorded with a spring loaded lever.

Nerve-sartorius preparation of frog :

Sciatic plexus in the lower abdomen was traced down to its branch to sartorius. The nerve and the sartorius muscle were removed from the frog. The nerve-sartorius preparation was mounted in an organ bath containing 50 *ml* of frog Ringer's solution (25°-27°C) and contractions to nerve stimulation were recorded by a spring loaded lever. The sartorius muscle removed from the opposite side was mounted in the second organ bath and contractions to direct stimulation were recorded.

Stimulation of nerve or muscle in all the experiments was by supramaximal rectangular pulses of 1 msec or 4 msec duration respectively every 10 sec.

Drugs :

Acetylcholine chloride, imipramine hydrochloride (Tofranil), carbaminoylcholine chloride (carbachol), caffeine and sodium benzoate, physostigmine salicylate, d-tubocurarine chloride, succinylcholine chloride, potassium chloride, calcium chloride, tetraethylammonium bromide, chlorpromazine hydrochloride and procaine hydrochloride were used; concentration refers to the salts. Imipramine solution was freshly prepared in distilled water.

RESULTS

Frog rectus abdominis :

Imipramine (1-50 $\mu g/ml$) had no effect on the acetylcholine sensitive recti of frog. Imipramine (2-6 $\mu g/ml$) however, markedly inhibited acetylchloline-induced contraction. The inhibition was dose-related and the dose-response curve for acetylcholine was shifted by imipramine to the right but non-competitively (Fig. 1).

The recovery from imipramine-induced inhibition was very slow and only partial (<30% in 1 hr). The inhibition by imipramine (5 $\mu g/ml$) was antagonised by physostigmine (5 $\mu g/ml$, n=4), and augmented by $CaCl_2$ (100 $\mu g/ml$, n=4), but remained unaltered by KCl (100 $\mu g/ml$, n=4). Inhibition of acetylcholine responses by procaine (150 $\mu g/ml$) was completely antagonised by physostigmine (5 $\mu g/ml$, n = 3).

Table I summarises the effect of imipramine on other spasmogen-induced contractions.

Imipramine (2 $\mu g/ml$) and tubocurarine (0.05 $\mu g/ml$) exhibited an additive effect (mean block due to tubocurarine plus imipramine, 40% ± 5 (±S.E.); mean block due to tubocurarine and imipramine alone, 21% ± 5 and 18% ± 2 respectively, n=4).

Nerve-muscle preparations :

The blocking activity of imipramine was expressed as (a) the onset (min) of the blocking effect, (b) the time (min) taken to produce 50% inhibition of the twitch height (T_{50}), and (c) the maximal % blockade produced (as percent of control). Table II summarises the neuromuscular blocking effect of imipramine on the rat phrenic nerve-diaphragm, the frog sciatic nerve-gastrocnemius and the nerve-sartorius preparations.

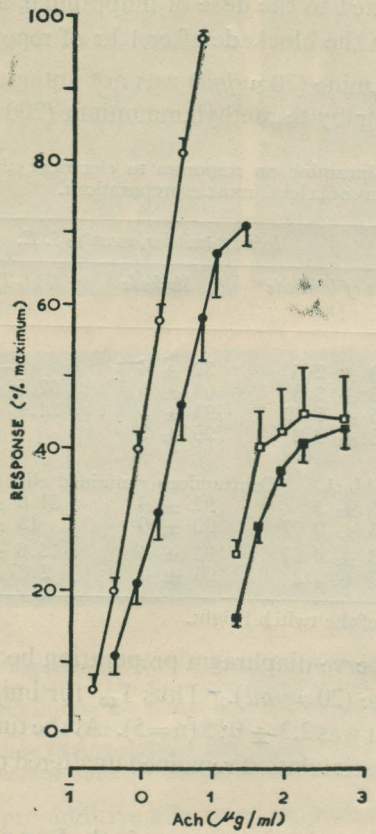

Fig. 1 : Frog rectus abdominis muscle. Cumulative log dose effect curves for acetylcholine (ACh) responses; control responses (0——0), and responses in presence of 2 μg/ml (●——●), 4 μg/ml (□——□) and 6 μg/ml (■——■) of imipramine. Vertical bars show S. E. of the means of 18 (control) and 6 (imipramine) experiments.

TABLE I : Rectus abdominis muscle of frog : inhibition of spasmogen-induced responses by imipramine. Values for a minimum of 4 experiments.

Spasmogen (μg/ml)	*Imipramine (μg/ml)*	*% inhibition*
Carbachol		
0.7	5	100
2.1	5	58–67
Succinylcholine		
15	5	100
45	5	40-55
KCl		
1500	10	No inhibition
Caffeine		
2500	10	No inhibition
Chlorpromazine		
25	10	No inhibition

In the rat phrenic nerve-diaphragm and the frog sciatic nerve-gastrocnemius preparations imipramine (5-20 $\mu g/ml$) first enhanced and then depressed markedly the contractile responses first to indirect and then to direct stimulation. Tetanus was poorly sustained and the contractions were further reduced following the tetanus (Fig. 2a). Enhanced responses were consistently obtained at 20 $\mu g/ml$ of imipramine, being less marked with lower doses. The duration of enhanced responses was inversely related to the dose of imipramine, and lasted from 15 min to 50 min. There was no recovery from the blockade after 1 hr of repeated wash.

The blockade produced by imipramine (20 $\mu g/ml$) was not antagonised by physostigmine (5 $\mu g/ml$), $CaCl_2$ and KCl (100 $\mu g/ml$), or by tetraethylammonium (200 $\mu g/ml$).

TABLE II : Effects of Imipramine on responses to electrical stimulation of isolated nerve-skeletal muscle preparations.

Preparation	*Imipramine ($\mu g/ml$)*	*Indirect twitches, mean ±S. E.*			*Direct twitches, Mean % blockade ±S.E.*
		Onset of blockade (min)	*% blockade*	T_{50}*	
Rat phrenic nerve-diaphragm	5 (n=3)	48.3 ± 2.6	32 ± 4	—	31 ± 2
	10 (n=4)	27.5 ± 3.3	68 ± 5	32.5 ± 4.24	57 ± 11
	20 (n=7)	13.6 ± 5.29	93 ± 3	22.4 ± 4.47	76 ± 8
	30 (n=3)	0.61± 0.2	96 ± 3	4 ± 0.8	70 ± 6
Frog sciatic nerve-gastrocnemius	5 (n=2)	No block	Contractions remained slightly augmented for 1 hr		
	10 (n=4)	35 ± 3	92 ± 4	51.8 ± 4.79	50 ± 6
	20 (n=4)	9.3 ± 0.67	90 ± 6	15 ± 8.4	42 ± 5
Frog nerve-sartorius	5 (n=4)	4.3 ± 0.67	82 ± 4	13.6 ± 0.88	66 ± 4
	10 (n=4)	1.19 ± 0.2	96 ± 3	2.87± 0.24	82 ± 5

*T_{50} = time (min) to produce 50% inhibition of the twitch height.

Partially curarized (0.05 $\mu g/ml$) nerve-diaphragm preparation became extremely sensitive to the blockade produced by imipramine (20 $\mu g/ml$). Thus T_{50} for imipramine alone was 22.4 ±4.47 (±S.E.) while with tubocurarine it was 2.3 ± 0.5 (n=5). At the time of complete blockade by imipramine the response to the direct stimulation remained unaltered or increased or decreased slightly (Fig. 2b).

Imipramine (5-10 $\mu g/ml$) rapidly blocked the responses in the frog nerve-sartorius to indirect and direct stimulaiton without prior augmentation. The time course for the inhibition to indirect and direct responses was parallel. The blockade persisted after 45 min of repeated wash. Physostigmine and KCl did not affect the blockade.

DISCUSSION

Marked antiacetylcholine effect of imipramine on the frog rectus observed in the present experiments seems to be nonspecific as evidenced by the behaviour of the dose-response curves for acetylchloine in presence of imipramine. Imipramine exhibits local anaesthetic action (10) and like inipramine procaine does not antagonise chlorpromazine (9) and potassium (8) induced contraction in skeletal muscle. Unlike procaine (9) however, imipramine did not inhibit caffeine-induced contracture. This might be so because like caffeine, imipramine has been

reported to produce contracture of single skeletal muscle fibre attributable to its action upon intracellular calcium storing structure (1), and caffeine partially restores the responses in rat diaphragm inhibited by desipramine (5).

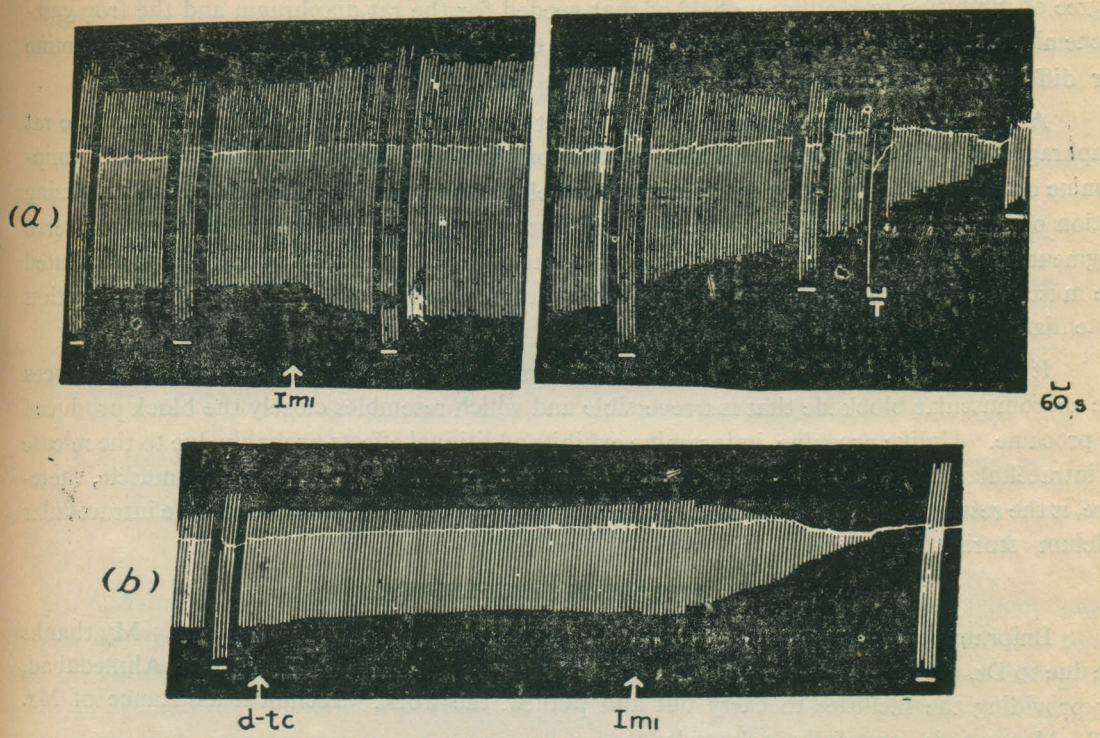

Fig. 2 : Rat phrenic nerve-diaphragm. Twitch responses to indirect stimulation (supramaximal rectangular pulses of 1 msec duration at 1/10 sec) and to direct muscle stimulation (supramaximal rectangular pulses of 4 msec duration at 1/10 sec) during the period indicated by horizontal bars. (a) Effect of imipramine (Imi, 20 $\mu g/ml$) on the indirect and direct responses. T, tetanus (50 Hz X 60 sec.) Break includes 12 min. (b) Partially curarized preparation (d-tubocurarine 0.05 $\mu g/ml$, added at d-tc) and effect of imipramine (Imi, 20 $\mu g/ml$) on it. Note the marked difference in the onset and progress of the blockade in (a) and (b).

Additive and supra-additive effects of imipramine with tubocurarine on the frog rectus and the rat diaphragm respectively should not be taken to indicate curare-like action of imipramine as procaine also reduced responses to single shocks in curarized muscle (7), and like procaine, imipramine-like drugs have been shown to decrease the release of neurotransmitter in the rat phrenic nerve-diaphragm (5). At the time of complete neuromuscular blockade by imipramine of the partially curarized preparation the direct contraction remained minimally affected indicating that the site of tubocurarine-imipramine interaction is the motor endplate and the prejunctional nerve terminals.

Physostigmine-imipramine antagonism seems to be nonspecific as it was observed only in the frog rectus where physostigmine effectively antagonised procaine-induced inhibition.

Apart from the difference in the time course there was no marked difference in the total blocking effect of imipramine on the indirect and direct stimulation in the rat diaphragm and

the frog sartorius preparations. The nonselectivity of the action of imipramine was most clearly observed in the frog sartorius where the total blockade as well as the time course was similar for the inhibition to indirect and direct stimulation. Moreover, the dose needed for the equal degree of inhibition was only one half of that needed for the rat diaphragm and the frog gastrocnemius preparations. This might be due to the difference in penetrability of imipramine for different nerve-muscle structures.

Augmented responses to indirect and direct stimulation prior to the blockade in the rat diaphragm and the frog gastrocnemius preparations may be due to the direct action of imipramine on the muscle probably by releasing intracellular calcium (1). Rapid onset of stabilizing action of imipramine leading to quick blockade may be responsible for the failure to observe augmented responses in the nerve-sartorius preparation. Chang and Chuang (5) have attributed the muscle stimulant effect of imipramine-like agents to the prolongation of the muscle action potential.

It is evident that imipramine, in the doses employed in the present experiments, produces the neuromuscular blockade that is irreversible and which resembles closely the block produced by procaine. Unlike procaine, imipramine exhibits additional effects probably due to the release of intracellular calcium. The final effect of imipramine on the isolated skeletal muscle, therefore, is the result of its membrane stabilizing action associated with its action on the intracellular calcium storing structure.

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