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Mechanism of MJ 1999-induced blockade of neuromuscular transmission

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MJ 1999 [4-(isopropylamino-1-hydroxyethyl)methanesulphonanilide hydrochloride] produced a dose-related blockade of indirectly evoked twitches of the rat diaphragm, without materially altering the effects of direct stimulation. The magnitude of blockade was comparable whether the indirect stimulation was at 1/s or 1/20 s. The blockade was less pronounced at 25° than at 34°. The blockade was antagonized by neostigmine, potassium chloride, succinylcholine, tetraethylammonium, adrenaline, isoprenaline and, to some extent, by noradrenaline. Blockade by MJ 1999 was additive to that due to tubocurarine; large concentrations of yohimbine, tolazoline, phenoxybenzamine and dihydroergotamine quickly antagonized MJ 1999 blockade. Small concentrations of adrenaline, isoprenaline, tolazoline or yohimbine prevented MJ 1999 blockade. MJ 1999 had no effect on nerve conduction. It is proposed that MJ 1999 has a two-fold mode of action at the neuromuscular junction; a curare-like effect and an action on β-receptors.

Sympathomimetic amines and the stimulation of sympathetic nerves can affect the skeletal neuromuscular system in many ways (see Bowman & Nott, 1969). There is strong evidence that β-adrenoreceptors are involved in the direct actions of adrenaline on the mammalian muscle. However, the effects of β-adrenoreceptor blocking agents on neuromuscular transmission have not been extensively examined. Pronethalol and propranolol, *in vivo* and *in vitro*, have yielded variable results (Morales-Aguilerá & Vaughan Williams, 1965; Türker & Kiran, 1965; Standaert, Levitt & Roberts, 1966; Wislicki & Rosenblum, 1967; Usubiaga, 1968). The neuromuscular blockade observed was ascribed to a local anaesthetic action of these agents, to depression of motor nerve terminals or to reduced output of the transmitter.

The β-adrenoreceptor blocker, MJ 1999 (Lish, Weikel & Dungan, 1965) has little local anaesthetic activity either in specific tests (Lish & others, 1965; Schmid & Calvin, 1967) or in neuronally evoked responses of smooth muscle (Bartlet & Hassan, 1969). It seemed worthwhile therefore to investigate this agent further for reported neuromuscular blocking effects (Standaert & Roberts, 1967).

METHODS AND MATERIALS

The rat phrenic nerve diaphragm preparation

The preparations were obtained from male Norwegian rats, 200 to 300 g. The bathing medium was Tyrode solution, g/litre: NaCl 8·0, KCl 0·2, $CaCl_2$ 0·2, $MgCl_2$ 0·1, Na_2HPO_4 0·05, $NaHCO_3$ 1·0 and dextrose, 2·0, maintained at 34° ± 1° and

gassed with 5% carbon dioxide in oxygen; pH after equilibration was 7·6. Stimulation was carried out alternately every 10 s by supramaximal pulses applied directly to the muscle (pulse width 5·0 ms) or to the nerve (pulse width 0·2 ms).

The effect of blocking drugs was examined by determining the latency period, $T\frac{1}{2}$ (the time to half-decay of twitch tension), and also the magnitude of blockade. These parameters were computed from the plot of percentage blockade (semi-logarithmic scale) against time (min) as described by Freeman (1968).

Method for local anaesthetic activity

In 5 experiments about 2·5 cm of phrenic nerve along with the stimulating electrodes was bathed in a chamber separated from the one which lodged the hemidiaphragm and the remaining portion of the nerve (Matthews & Quilliam, 1964).

Drugs. The drugs used were (+)-tubocurarine chloride, neostigmine methylsulphate, choline chloride, succinylcholine chloride, tetraethylammonium bromide, yohimbine hydrochloride, tolazoline hydrochloride, phenoxybenzamine hydrochloride, dihydroergotamine methane-sulphonate, (−)-adrenaline (Sigma Chemical Co.), (±)-noradrenaline hydrochloride (Sigma Chemical Co.), (±)-isoprenaline hydrochloride (Fluka AG) and MJ 1999 [4-(isopropylamino-1-hydroxyethyl) methanesulphonanilide hydrochloride, Mead Johnson & Co.]. Doses of adrenaline, noradrenaline and isoprenaline refer to the base and of the other compounds to the salts.

RESULTS

Effect of MJ 1999 on the responses to electrical stimulation

MJ 1999 (6·25 to 25 μg/ml) reduced indirectly evoked twitches of the rat diaphragm; reduction of direct twitches was moderate and seen only in higher concentrations (Table 1). The regression of mean maximal blockade of indirect twitches on log dose of MJ 1999 was linear and highly significant ($P < 0·001$). With each concentration of MJ 1999 the decline of twitch height was exponential. MJ 1999 (30·0 μg/ml) produced a mean blockade of 90%; the mean latency period and $T\frac{1}{2}$ were 2·3 and 5·2 min, respectively (n = 16, Table 1). Using this as test concentration it was found

Table 1. *Rat phrenic nerve diaphragm preparation. Effect of MJ 1999 on electrical stimulation.*

Experimental conditions*	MJ 1999 (μg/ml)	n =	Indirect twitches. Mean ± s.e.			Direct twitches. Mean % blockade ± s.e.
			Latency period (min)	% Blockade	Time to half-decay (min)	
1. Nil (control)	6·25	5	3·4 ± 0·3	27·0 ± 2·1	—	7·0 ± 5·0
	12·5	5	2·6 ± 0·2	54·5 ± 3·0	—	15·5 ± 3·5
	25·0	8	2·5 ± 0·2	80·0 ± 1·3	5·3 ± 0·6	18·5 ± 4·2
	30·0	16	2·3 ± 0·3	90·0 ± 3·2	5·2 ± 0·7	20·0 ± 2·5
2. Indirect stimulation at 1/s	30·0	5	3·1 ± 0·4	85·5 ± 3·8	5·8 ± 1·1	—
3. Bath temperature 25°	30·0	5	4·4 ± 0·9†	65·0 ± 5·0†	11·1 ± 1·2†	17·0 ± 1·5
4. In presence of						
adrenaline (60 ng/ml)	30·0	4	—	0·0†	—	0·0
isoprenaline (60 ng/ml)	30·0	3	—	0·0†	—	0·0
tolazoline (7 μg/ml)	30·0	4	10·2 ± 3·0†	10·0 ± 2·0†	—	0·0
yohimbine (5 μg/ml)	30·0	3	8·3 ± 3·6†	7·0 ± 4·0†	—	0·0

* Tyrode solution at 34° C, gassed with 5% carbon dioxide in oxygen, pH 7·6; stimulation, supramaximal pulses, direct (width 5·0 ms) and indirect (width 0·2 ms) alternated at 10 s intervals.
† Differs significantly ($P < 0·05$) from the effect of 30 μg/ml MJ 1999.

that consecutive exposures to MJ 1999, separated by 30 min wash periods, gave reproducible data with up to 5 exposures without tachyphylaxis. The blocking activity of MJ 1999 was similar whether stimulation was at 1/s or at 1/20 s (Table 1) and was markedly reduced at a bath temperature of 25°.

Effects of various drugs on MJ 1999 *blockade*

The blocking activity of MJ 1999 (30 μg/ml), like that of tubocurarine (1·0 μg/ml), was antagonized by neostigmine, KCl, succinylcholine, choline and by tetraethylammonium (Table 2). Also, MJ 1999 and tubocurarine had an additive action (mean block due to MJ 1999, 6·26 μg/ml with tubocurarine, 0·3 μg/ml was 43%; mean block due to tubocurarine alone, 22%; n = 4).

Table 2. *Antagonism of MJ* 1999- *and tubocurarine-induced blockade of indirectly induced contractions of rat diaphragm by various drugs.* The test dose of MJ 1999 (30·0 μg/ml) and of tubocurarine (1·0 μg/ml) was such that it produced over 90% blockade of indirect twitches. Values are means from a minimum of 4 experiments.

Drug	Concentration (μg/ml)	Mean % antagnoism of blockade MJ 1999	Tubocurarine
Neostigmine	2·0	62	68
KCl	5·0	48	56
Succinylcholine	5·0	45	35
Choline	100·0	51	60
Tetraethylammonium	200·0	70	78
Isoprenaline	0·3	80	0
Adrenaline	0·3	72	50
Noradrenaline	0·6	40	35
Yohimbine	30·0	100	0
Tolazoline	30·0	80	0
Phenoxybenzamine	30·0	74	0
Dihydroergotamine	30·0	68	0

In view of the similarities of blockade by MJ 1999 and tubocurarine, it was of interest to examine if the two agents further resembled one another in their interaction with sympathomimetic amines. Table 2 shows that tubocurarine blockade was antagonized by adrenaline and to a lesser degree, by noradrenaline; isoprenaline was ineffective. Furthermore, the anticurare action of adrenaline was blocked by tolazoline (5·0 μg/ml) added 2–4 min before addition of adrenaline (n = 4; Fig. 1B). On the other hand, MJ 1999 blockade was quickly antagonized by adrenaline as well as by isoprenaline; noradrenaline was partly effective (Table 2). In the presence of small concentrations of adrenaline or isoprenaline, MJ 1999 did not exhibit any blocking activity (Table 1). The antagonistic action of adrenaline to MJ 1999 was not significantly affected by tolazoline (n = 4; Fig. 1A).

In experiments in which the effect of tolazoline on the antagonistic action of adrenaline to MJ 1999 was examined, it was found that if the concentration of tolazoline was increased, there was a reversal of MJ 1999 blockade even before the addition of adrenaline. Further observations (Table 2) showed that all the four α-adrenoreceptor blockers studied, yohimbine, tolazoline, phenoxybenzamine and dihydroergotamine, produced a quick and near complete reversal of MJ 1999 blockade whereas they had no effect on the blockade produced by tubocurarine.

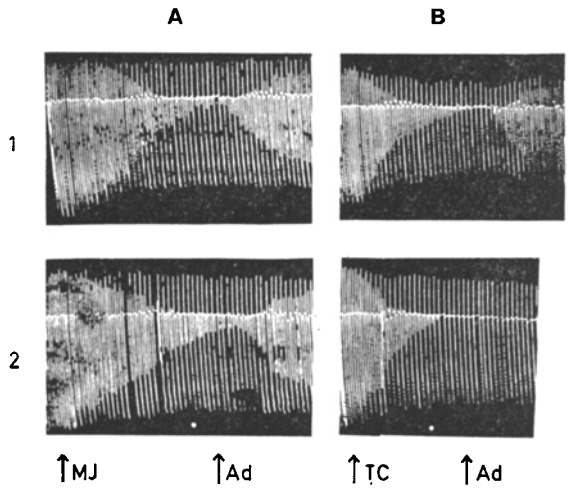

FIG. 1. Rat phrenic nerve diaphragm preparation. Tyrode solution, 34°, gassed with 5% CO_2 in O_2. Alternate direct (pulse width, 5·0 ms) and indirect (pulse width, 0·2 ms) supramaximal stimulation at 10 s intervals. MJ 1999 (30 μg/ml, at MJ) and tubocurarine (1·0 μg/ml, at TC) blocked indirect twitches (in A and B, respectively). Adrenaline (0·3 μg/ml, at Ad) antagonized the blockade (A–1 and B–1). Tolazoline (5·0 μg/ml, at white dot) added before adrenaline did not counteract the anti-MJ 1999 effect of adrenaline (A–2) but it abolished anti-curare effect of this drug (B–2).

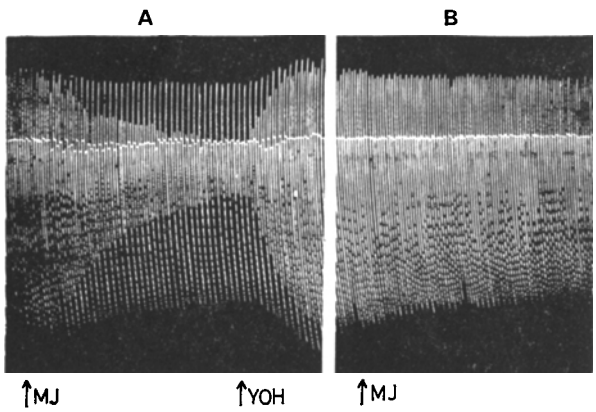

FIG. 2. Rat phrenic nerve-diaphragm preparation (see Fig. 1 for experimental conditions). A. Blockade of indirect twitches by MJ 1999 (30 μg/ml, at MJ) and its reversal by yohimbine (30 μg/ml, at YOH). B. MJ1999 had no blocking effect when bath fluid contained yohimbine (5·0 μg/ml).

Moreover, previous administration of small concentrations of either yohimbine or tolazoline could prevent blockade due to MJ 1999 (Table 1). Fig. 2 shows the interaction between MJ 1999 and yohimbine.

Effect of MJ 1999 *on nerve-conduction*

MJ 1999 (50 μg/ml) added to the "nerve bath" had no effect on indirectly induced contractions of the diaphragm over at least 40 min.

DISCUSSION

The blockade of indirect twitches due to MJ 1999 does not appear to be due to reduced efficiency of the neurosecretory process. MJ 1999 had no effect on axonal conduction and, though this in itself does not rule out reduced impulse traffic at the terminal nerve endings, such an effect is unlikely as MJ 1999 is free of membrane stabilizing activity. Moreover, the agent, unlike pronethalol or propranolol, has no effect on succinylcholine-induced repetitive firing, fasciculation or augmented twitch tension (Usubiaga, 1968). However, the nature of the blocking activity, as well as the antagonistic effect of various drugs which either increase the efficiency of the neurosecretory process or increase the effect of released transmitter, suggests that MJ 1999 has a tubocurarine-like mode of action.

Our results also suggest that MJ 1999 has a second component of action on β-adrenoreceptors. Thus, the rank order of potency of sympathomimetic amines as antagonists of MJ 1999 and tubocurarine was different. Isoprenaline antagonized MJ 1999 blockade. Furthermore, the antagonistic action of adrenaline to MJ 1999, unlike the anticurare activity of this agent, was not influenced by tolazoline and hence does not seem to be mediated through α-adrenoreceptors. The antagonism of MJ 1999, but not of tubocurarine, by larger concentrations of α-adrenoreceptor blocking agents is a further point of interest. The antagonism of α-adrenoreceptor blocking agents by β-adrenoreceptor blockers at the smooth muscle effector is well documented (Hull, Elthrington & Horita, 1960; Olivers, Smith & Anorow, 1967; Yamamura & Horita, 1968) and is generally believed to be due to a phenomenon of displacement at the receptor level. The reverse type of antagonism, that is, of β- by the α-adrenoreceptor blockers has not yet been reported but a similar displacement at receptor level could well be implicated.

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