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Inhibition by propranolol of the contractile response of the rat diaphragm to tetanic field stimulation *in vitro*

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1 Contraction of the rat isolated diaphragm in response to maximal tetanic stimulation was examined before and after isoprenaline or propranolol.

2 Isoprenaline (10^{-4} M) did not affect maximum isometric force, whereas propranolol depressed maximum force in a concentration-dependent manner (10^{-6}–10^{-4} M).

3 Inhibition due to propranolol (10^{-4} M) could not be overcome by increasing the intensity or duration of electrical stimulation, and was only partially reversed (mean 73% ± 10 s.e.mean) after washing.

4 Pretreatment with isoprenaline did not alter the response of the muscle to propranolol, nor did neuromuscular blockade with (+)-tubocurarine.

5 The response to either stereoisomer of propranolol was similar to that obtained with the racemate.

6 Atenolol, a β-adrenoceptor blocking agent without membrane stabilizing activity, had minimal (< 10%) depressant effects on diaphragmatic force development.

7 Lignocaine (8.5×10^{-6}–8.5×10^{-5} M) produced a concentration-related decrease in isometric force, similar to that with propranolol.

8 It is concluded that propranolol decreases the contractile force of the rat isolated diaphragm by a mechanism related to stabilization of excitable membranes.

Introduction

Although β-adrenoceptor agonists and antagonists are widely used in the treatment of cardio-pulmonary disease, little is known regarding the effects of these agents on respiratory skeletal muscle function, especially during conditions of increased respiratory work. That β-adrenoceptive drugs might have effects on the contractile function of the diaphragm is suggested by studies showing (a) an abundance of β-adrenoceptors in diaphragm, (Bowman & Raper, 1964; Lands, Ludena & Buzzo, 1967), (b) alteration of diaphragmatic metabolic activity by drugs that act on adrenoceptors (Bowman & Raper, 1964; Dhalla, 1966; Molme-Lundholm, Svedmyr & Vamos, 1967) and (c) potentiation of diaphragmatic twitch response by isoprenaline and adrenaline (Ritchie, 1952; Ellis & Beckett, 1954; Goffart & Lands *et al.*, 1967). Although muscle twitch response (force developed following a single electrical stimulus of approximately 1.0 ms in duration) (Brown, Bülbring & Burns, 1948; Lands *et al.*, 1967; Harry, Linden & Snow, 1974) has previously been used to assess the effects of drugs that interact with adrenoceptors on contractions of the diaphragm (Bülbring, 1946), it seemed unlikely that the twitch response would resemble the activity of diaphragm *in vivo*. The diaphragm in man, for example, is normally activated by a phasic discharge lasting several seconds, and maximum development of force requires a stimulus frequency of 100 Hz in the phrenic nerve (Moxham, Morris, Spiro, Edwards & Green, 1981). We therefore examined the effects of β-adrenoceptive drugs on diaphragm during high frequency, tetanic stimulation.

Methods

Muscle preparation and apparatus

Male Sprague-Dawley rats weighing 150–300 g were stunned by a blow on the head and exsanguinated. The thoracic and abdominal cavities were opened and a strip from the left hemidiaphragm (2–4 mm × 15–20 mm) was removed, using incisions

parallel to the direction of the muscle fibres. Portions of central tendon and rib cage were excised with the strip for use as points of attachment. Strips were mounted vertically in 10 ml tissue chambers maintained at 37°C by an external circulating bath (Haake, Model FS) and attached to a transducer for measurement of isometric force (Grass Instruments, Model FTO.3C). Resting muscle length could be altered by raising or lowering the force transducer to produce a resting force of 250 mg, a value maintained throughout each experiment by making small adjustments (total < 0.5 mm), which did not affect the maximum active force. The output of the transducer was amplified (G.M.G. Scientific, Model 884 amplifier) and recorded with a Harvard Apparatus (Model 350) pen recorder. Platinum wire electrodes (15×1.0 mm) were positioned parallel to the muscle strips and connected to a stimulator (Grass Instru-

Table 1. **A** Effect of propranolol on maximal force of contraction of rat isolated diaphragm

		Force (g)			
		0 min†	2 min	5 min	10 min
Untreated control		10.4	10.5	10.5	10.2
$n=7$		(1.0)	(1.0)	(0.9)	(0.8)
	%	100	101	101	98
Propranolol concentration					
(10^{-6} M)		10.2	10.1	9.8	9.7
	s.e.mean	(1.7)	(1.7)	(1.8)	(2.0)
$n=4$	%	100	99	96	95
(10^{-5} M)		6.8	5.2*	4.3*	3.5*
	s.e.mean	(0.7)	(0.7)	(0.6)	0.4)
$n=7$	%	100	76	63	51
(5×10^{-5} M)		4.7	2.2*	1.1*	0.9*
	s.e.mean	(0.6)	(0.5)	(0.3)	(0.3)
$n=4$	%	100	47	23	19
(10^{-4} M)		12.0	5.4*	2.0*	0.6*
	s.e.mean	(1.3)	(0.5)	(0.2)	(0.1)
$n=6$	%	100	45	17	5
(10^{-5} M)		4.8	2.8	1.9	
+					
isoprenaline (10^{-3}–10^{-4} M)	s.e.mean	(0.6)	(0.85)	(0.9)	
$n=5$	%	100	59	49	

B *Effect of propranolol (10^{-5} M) with frequent and infrequent stimulation*

		Force (g)	
		0 min	10 min
Stimulation			
1 per min		7.7	3.5*
	s.e.mean	(2.9)	(2.8)
$n=5$	%	100	45
1 per 10 min		9.7	4.0*
	s.e.mean	(2.5)	(1.4)
$n=5$	%	100	41

(A) '2, 5 and 10 min' refer to force developed at those time intervals after addition of propranolol. Strips were stimulated maximally every min. n = number of strips, s.e.mean = standard error of the mean, % = % of baseline force. * indicates a significant difference from 0 min, $P < 0.005$.

(B) same as **(A)**, except that, after 0 min stimulation, the lower group (stim rate = 1 per 10 min) were not stimulated again until 10 min after propranolol.

† Variation among groups in mean force at time 0 was due to differences in the size of the muscle strips (10^{-4} M + 10^{-6} M strips = 4×20 mm; 10^{-5} M + 5×10^{-5} M were 2×15 mm).

ments, Model S44). Tissues were bathed in Tyrode solution (mM: NaCl 139.2, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.4, NaH_2PO_4 0.42, $NaHCO_3$ 11.3 and dextrose 11) (Bülbring, 1946) and oxygenated continuously with a mixture of 95% O_2, and 5% CO_2. Drugs were prepared within 30 min before administration using powdered form dissolved in Tyrode solution. The agents used in these experiments were: (+)-tubocurarine chloride (Lilly, Indianapolis), (±)-propranolol, (+)-propranolol, (−)-propranolol (Ayerest, New York), atenolol (Stuart, Wilmington), lignocaine hydrochloride (Elkin-Sinn, Cherry Hill) and isoprenaline hydrochloride (Sigma, St. Louis).

Experiments

Preliminary studies revealed that maximum tetanic force was produced with a stimulus of 50 V, 120 Hz, 0.2 ms pulse duration, with a train duration of 3 s. All results were obtained with stimuli of this magnitude unless otherwise specified. Prior to study, strips were stimulated at 1 min intervals until stable contractions were obtained, usually within 7–15 min. Each diaphragm strip served as its own control. Differences were determined by paired *t* test analysis; $P<0.05$ was considered to be significant. Values in the text refer to mean ± s.e.mean of *n* determinations.

During studies with isoprenaline, strips were stimulated at rates of either 0.1 or 1.0 per min, and the mean values of three such stimuli before and after isoprenaline were recorded. In studies with racemic propranolol, stereoisomers of propranolol, atenolol, and lignocaine, the immediate pre-drug force values were compared with those subsequently measured at 2, 5 and 10 min after the drug.

Results

Stability of untreated strips and response to isoprenaline

The response of untreated strips to repetitive tetanic stimulation is presented in Table 1. Contraction following this stimulation (3 s trains once per min) were consistent (< 4% variation, $n=7$).

Isoprenaline (10^{-5}–10^{-4} M) did not affect maximal force generation, irrespective of whether stimulation was performed every 10 min (8.7 ± 2.4 g, $n=7$, before isoprenaline; 8.6 g ± 2.5, $n=7$, after isoprenaline, $P>0.05$) or every min (10.5 ± 2.6 g, $n=4$, before; 10.5 ± 2.5 g, $n=4$, after, $P>0.05$). Resting force was also unchanged by isoprenaline.

Propranolol

Propranolol (10^{-6}–10^{-4} M) reduced maximal tetanic force in a concentration-dependent manner (Table 1). A depressant effect with 10^{-6} M propranolol was seen in only 2 strips, while concentrations of 10^{-5} M or greater markedly reduced force in every strip, indicating a threshold concentration for this effect between 10^{-6} M and 10^{-5} M. The reduction in force of contraction was not overcome by increasing stimulus voltage, frequency or duration. The decrease in force 10 min after propranolol was not significantly different whether or not strips were stimulated during this interval (Table 1B). The depressant effect of propranolol was slowly and only partially reversed after 45 min of washing with 200 ml of drug-free Tyrode solution (mean recovery 73 ± 10%, $n=6$). Similar effects of propranolol were also observed in several

Table 2 **A** Effect of tubocurarine on maximal force of contraction of rat isolated diaphragm

	0 min (0.2 ms)	*Tubocurarine* (0.2 ms)	*Tubocurarine* (2.0 ms)
$n=6$			
Force (g)	10.9	5.1*	10.4
s.e.mean	1.8	1.4	1.3
%	100	47	95

B Effect of propranolol in curarized diaphragm (2.0 ms pulse duration)

	0 min	2 min	5 min	10 min
$n=5$				
Force (g)	6.6	4.7*	3.3*	2.3*
s.e.mean	0.8	0.7	0.5	0.3
%	100	71	50	35

A, B concentration of tubocurarine = 6 $\mu g\,ml^{-1}$. Stimulus pulse durations of 0.2 ms or 2.0 ms are specified. s.e.mean = standard error of the mean. * signifies a value significantly different from baseline ($P<0.005$). *n* = number of strips. With stimulus pulse duration increased to 2.0 ms, force output returns to normal despite neuromuscular blockade, due to enhanced direct membrane stimulation.

strips during submaximal electrical stimulation (voltage reduced from 50V to 10–20V).

Propranolol and isoprenaline

To determine if the inhibiting effect of propranolol could be abolished or reduced by a competitive β-adrenoceptor agonist, isoprenaline (10^{-3}M–10^{-4}M) was added before propranolol (10^{-5}M) in 5 strips. Isoprenaline did not affect the response to propranolol (Table 1).

Propranolol and tubocurarine

The muscle and the nerve endings will be depolarized by field stimulation. To examine the possibility that propranolol might be exerting its depressant effects by blocking the component of stimulation occurring at neuromuscular junctions, six diaphragm strips were studied following the addition of (+)-tubocurarine. Tubocurarine (8.8×10^{-6}M) decreased mean maximal force by about half (no greater effect was seen with up to 8.8×10^{-5}M tubocurarine). By increasing stimulus pulse duration from 0.2 ms to 2.0 ms, the contractions returned to their previous level, presumably due to increased direct muscle stimulation (Baraka, 1974). Subsequently, despite the presence of tubocurarine, propranolol exerted its usual depressant action (Table 2B).

Propranolol stereoisomers and atenolol

Since high concentrations of isoprenaline did not block the depressant effects of propranolol on the diaphragm, it seemed unlikely that this effect was due

Table 3 Effect of stereoisomers of propranolol and of atenolol on maximal force of contraction of rat isolated diaphragm

Drug		*Froce* (g) 0 min	2 min	5 min	10 min
Untreated control		10.4	10.5	10.5	10.2
$n=7$	s.e.mean	(1.0)	(1.0)	(0.9)	(0.8)
	%	100	101	101	98
(−)-Propranolol (10^{-5}M)		10.5	9.7	8.8*	7.2*
$n=9$	s.e.mean	(1.5)	(1.4)	(1.3)	(1.1)
	%	100	92	84	69
(−)-Propranolol (10^{-4}M)		7.2	3.5*	1.3*	0.5*
$n=9$	s.e.mean	(1.2)	(0.5)	(0.2)	(0.1)
	%	100	49	18	7
(+)-Propranolol (10^{-5}M)		9.3	8.0*	6.8*	4.9*
$n=7$	s.e.mean	(1.8)	(1.4)	(1.0)	(0.6)
	%	100	86	73	53
(+)-Propranolol (10^{-4}M)		4.9	2.0*	0.7*	0.3*
$n=7$	s.e.mean	(0.6)	(0.3)	(0.1)	(0.02)
	%	100	41	14	6
Atenolol (10^{-4}M)		8.5	8.4	8.2	7.8**
$n=10$	s.e.mean	(0.9)	(0.8)	(0.8)	(0.7)
	%	100	99	96	92
Lignocaine (8.5×10^{-5}M)		9.7	4.2*	4.0*	3.4*
$n=6$	s.e.mean	(1.2)	(0.7)	(0.6)	(0.4)
	%	100	43	41	35
Lignocaine (4.25×10^{-5}M)		8.8	5.2*	4.5*	3.9*
$n=5$	s.e.mean	(0.5)	(0.6)	(0.4)	(0.4)
	%	100	59	51	44
Lignocaine (8.5×10^{-6}M)		10	8.3	7.5*	6.1*
$n=6$	s.e.mean	(1.0)	(0.8)	(0.7)	(0.6)
	%	100	83	75	61

n = number of strips, s.e.mean = standard error of the mean, % = percentage of mean 0 min (pre-drug) force.
* Signifies a value significantly different from 0 min with $P<0.005$.
** Refers to $P<0.02$.

to β-adrenoceptor blockade. As the membrane stabilizing actions of racemic propranolol might be responsible for the reduction in force, further studies were performed with (−)-propranolol (β-adrenoceptor blockade and membrane stabilization), (+)-propranolol (membrane stabilization but little β-adrenoceptor blocking action) (Parmley & Braunwald, 1967), and atenolol (β-adrenoceptor blockade but no membrane stabilizing activity) (Brown, Carruthers, Johnston, Kelly, McAinsh, McDevitt, & Shanks, 1976). The results are presented in Table 3. Both stereoisomers of propranolol (10^{-5} M) caused marked reduction in maximum force within 10 min of drug addition. The reduction at 2 and 5 min after the drug was somewhat less than that observed with racemic propranolol, especially when using the (−)-isomer. Subsequent addition of either stereoisomer in high concentration (10^{-4} M) resulted in rapid and profound reduction in force, comparable to that observed with 10^{-4} M racemic propranolol. Atenolol, a beta-adrenoceptor blocking agent lacking membrane stabilizing activity, produced a small reduction, only at a high concentration (10^{-4} M) (Table 3) which was less ($P < 0.001$) than that following any form of propranolol.

Lignocaine

Lignocaine (8.5×10^{-5}–8.5×10^{-6} M) depressed diaphragmatic contractions in a concentration-dependent manner (Table 3), similar to that previously observed with propranolol.

Discussion

Isoprenaline had no effect on the force developed in response to intermittent, repetitive tetanic stimulation, which makes it unlikely that the depressant effect of propranolol followed β-adrenoceptor blockade. Furthermore, propranolol's depressant actions became apparent only at concentrations very much higher than that necessary for β-adrenoceptor blockade in rat isolated diaphragm (Dhalla, 1966) or myocardium (Barrett & Cullum, 1968). Finally, the inhibition was nearly identical when using equimolar concentrations of either racemic propranolol or the (+)-stereoisomer of propranolol, the latter of which has little β-adrenoceptor blocking activity.

Catecholamines are known to affect neuromuscular transmission. In a field stimulated preparation, muscle action potentials arise from excitation at neuromuscular junctions as well as from direct membrane depolarization (Baraka, 1974). In the present experiments, both modes of activation contribute to the response, as shown by the reduction (but not abolition) of the contractions after tubocurarine. During neuromuscular blockade, increasing stimulus intensity tenfold (pulse duration changed from 0.2 ms to 2.0 ms) resulted in sufficient membrane stimulation alone to return maximum force to normal. In the presence of tubocurarine, propranolol depressed contractions of the diaphragm to an extent equivalent to that in its absence. Thus the inhibition seen with propranolol is unlikely to be due to a depressant effect of propranolol at the neuromuscular junction.

Propranolol could also have affected cellular metabolism, leading to decreased contractility secondary to cellular energy depletion. Adrenaline increases ATP and creatinine phosphate levels in rat diaphragm (Molme-Lundholm *et al.*, 1967), stimulating glycogenolysis by increasing the rate of conversion of phosphorylase b to phosphorylase a, an effect blocked by propranolol (Dhalla, 1966). These effects of catecholamines, however, are believed to be mediated by β-adrenoceptors, making their relevance to the effects of propranolol seen here uncertain.

That the membrane stabilizing effect of propranolol may be the basis for propranolol-induced depression of force finds support in that (a) atenolol, a β-adrenoceptor blocking agent without membrane stabilizing activity, produced only minimal reduction in diaphragmatic force, even at high concentrations, and (b) the local anaesthetic lignocaine depressed diaphragmatic force in a manner similar to propranolol. Alternatively, propranolol may act by decreasing the release of calcium from the sarcoplasmic reticulum during stimulation. An effect of this type has been suggested as the mechanism of action of β-adrenoceptor agonists on contractility in other skeletal muscles (Holmberg & Waldeck, 1980).

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