EFFECTS OF PROSTAGLANDINS AND INDOMETHACIN ON NEUROMUSCULAR BLOCKING AGENTS

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ABSTRACT

The effects on neuromuscular blockade by d-tubocurarine and succinylcholine of inhibition of prostaglandin biosynthesis by indomethacin and of intra-arterial administration of prostaglandins E_2 and $F_{2\alpha}$, before and after inhibition of prostaglandin biosynthesis, were evaluated in the cat sciatic-tibialis preparation.

Non-specific inhibition of prostaglandin biosynthesis by indomethacin $3\text{ mg}\cdot\text{kg}^{-1}$ did not alter latency, maximal blockade or duration of neuromuscular blockade induced by d-tubocurarine or succinylcholine.

Prostaglandin E_2 antagonized twitch height depression by d-tubocurarine by an average of six per cent before and by 15 per cent after indomethacin, but potentiated the neuromuscular block of succinylcholine by an average of five per cent before and 60 per cent after indomethacin. Prostaglandin $F_{2\alpha}$ antagonized d-tubocurarine neuromuscular block by an average of 10 per cent before and 18 per cent after indomethacin, but potentiated succinylcholine block by an average of four per cent before and 12 per cent after indomethacin.

These results suggest that non-specific inhibition of prostaglandin biosynthesis alone does not influence d-tubocurarine or succinylcholine induced neuromuscular blockades. However, both prostaglandin E_2 and $F_{2\alpha}$ may induce transmitter release at the neuromuscular junction that may be enhanced by indomethacin, thus antagonizing the non-depolarizing blockade of d-tubocurarine and potentiating the depolarizing blockade of succinylcholine.

THE PROSTAGLANDINS are a group of compounds, named by von Euler in 1937, which possess varied pharmacological actions on the autonomic nervous system.¹ While the major area of investigation of prostaglandin action has been the cardiovascular system, little has been done on the effects of prostaglandins on the neuromuscular junction. The clinical use of non-steroidal anti-inflammatory agents such as aspirin, indomethacin and ibuprofen are common. They also inhibit the biosynthesis of prostaglandins.² The present study evaluates the effects of two prostaglandins, PGE_2 and $PGF_{2\alpha}$, and a prostaglandin synthesis inhibitor, indomethacin, and the interaction of indomethacin with PGE_2 or $PGF_{2\alpha}$ on the neuromuscular blocking action of d-tubocurarine and succinylcholine.

METHODS

Forty cats of either sex weighing 2.6 to 5.1 kg were anaesthetized with intraperitoneal alpha-

chloralose $100\text{ mg}\cdot\text{kg}^{-1}$. Following intubation of the trachea mechanical ventilation with air maintained arterial PCO_2 at 4.0 to 4.5 kPa (30–35 torr) and PO_2 at 11.3 to 12.7 kPa (85–95 torr). The tendon of the right anterior tibialis muscle was dissected free, sectioned near the point of osseous attachment and connected to a Grass FT-10 force displacement transducer. The right sciatic nerve was then sectioned and the distal peroneal nerve was isolated and supramaximal stimuli (1–8 volts) of 0.5 msec duration and 0.2 Hz were applied from a Grass stimulator, model S4G, through shielded platinum electrodes. The force of muscle contraction was recorded continuously on a Grass model 5 polygraph. The twitch tension exhibits only five per cent reduction from control over a three-hour period. The right femoral artery was exposed for intra-arterial drug administration. Body temperature was monitored and maintained at 36–38°C. The left femoral artery was cannulated and connected to a Statham P23 Db arterial pressure transducer for continuous pressure and blood gas monitoring to ascertain cardiopulmonary stability.

Eight animals (Group 1) received an intravenous bolus injection of d-tubocurarine $0.2\text{ mg}\cdot\text{kg}^{-1}$, followed by a continuous infusion of d-tubocurarine $100\text{ }\mu\text{g}/\text{ml}$, through a Harvard infusion pump to produce 90 per cent twitch height depression. The 90 per cent twitch height

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TABLE I
NEUROMUSCULAR BLOCKING ACTION OF D-TUBOCURARINE AND SUCCINYLCHOLINE
BEFORE AND AFTER INHIBITION OF PROSTAGLANDIN BIOSYNTHESIS BY INDOMETHACIN
(MEAN $\pm$ S.E., N = 4)*

	Latency (sec)		Percent of control in twitch depression		Time (min) to return to 90% control	
	Before	After	Before	After	Before	After
Tubocurarine						
0.1 mg $\cdot$ kg ⁻¹	106 ± 10.6	98 ± 8.7	76 ± 4.5	80 ± 2.7	16.0 ± 1.7	16.4 ± 1.5
0.2 mg $\cdot$ kg ⁻¹	61 ± 4.7	62 ± 5.0	96 ± 3.8	95 ± 1.5	25.5 ± 2.5	24.6 ± 2.7
Succinylcholine						
0.03 mg $\cdot$ kg ⁻¹	30 ± 3.0	26 ± 2.2	91 ± 2.7	93 ± 1.2	8.4 ± 0.8	8.6 ± 0.6
0.06 mg $\cdot$ kg ⁻¹	12 ± 1.6	13 ± 1.7	100 ± 0	100 ± 0	15.4 ± 2.7	16.1 ± 3.0

*No significant differences between before and after data.

depression was maintained for 20 minutes before proceeding. A slow (5 minute) intra-arterial injection of either prostaglandin E₂ 0.5 mg (PGE₂) (four animals), or F_{2 α} 0.5 mg (PGF_{2 α}) (four animals), was then given and the twitch height response was recorded. Another eight animals (Group 2) were treated the same as Group 1, except that indomethacin 3 mg $\cdot$ kg⁻¹ was given intravenously one hour before administration of the same dose of PGE₂ (four animals) or PGF_{2 α} (four animals). Indomethacin powder was dissolved by titration with 0.1 N Na₂CO₃ after the method of Wilkerson and Glenn.³

Another eight animals (Group 3) received an intravenous bolus of succinylcholine 0.03–0.1 mg $\cdot$ kg⁻¹ adequate to depress twitch tension 60 to 80 per cent. During maximal twitch tension depression, 0.5 mg of either PGE₂ (four animals) or PGF_{2 α} (four animals), was administered intra-arterially and the twitch height response recorded as above. Group 4 of eight animals were treated and studied in the same manner as Group 3, except that indomethacin, 3 mg $\cdot$ kg⁻¹ was given intravenously one hour before administration of the prostaglandins.

In another eight animals (Group 5) four cats received d-tubocurarine 0.1 and 0.2 mg $\cdot$ kg⁻¹ intravenously and four cats received succinylcholine, 0.03 and 0.06 mg $\cdot$ kg⁻¹ intravenously with the following parameters of neuromuscular function recorded: latency, maximal blockade obtained and time required for recovery to 90 per cent of control twitch tension. Methodology employed has been previously reported.⁴ A 45-

minute period was allowed between the two doses of the neuromuscular blocking agent. The same animal was studied one week later as described, except that indomethacin 3 mg $\cdot$ kg⁻¹ was given intravenously one hour before administration of the neuromuscular blocking agents.

Statistical significance was calculated using Student's t-test for paired or unpaired data. Differences with a probability of 0.05 or less were considered significant.

RESULTS

The latency period, maximum percent block obtained and time required to return to 90 per cent control twitch tension of neuromuscular blockade induced by d-tubocurarine and succinylcholine were not significantly altered by inhibition of prostaglandin biosynthesis induced by indomethacin (Table I). In these animals, the administration of the indomethacin vehicle, (0.1 N Na₂CO₃) produced no change in the control twitch tension.

Prostaglandin E₂ antagonized neuromuscular blockade induced by d-tubocurarine by an average of 6 per cent ($p > 0.05$) before and 15 per cent ($p < 0.05$) after indomethacin but potentiated neuromuscular block induced by succinylcholine by an average of five per cent ($p > 0.05$) before and 10 per cent ($p < 0.05$) after indomethacin (Table II).

PGF_{2 α} antagonized neuromuscular blockade by d-tubocurarine by an average of 10 per cent ($p < 0.05$) before and 18 per cent ($p < 0.05$)

TABLE II
EFFECTS OF PROSTAGLANDIN E_2 AND $F_{2\alpha}$ ON NEUROMUSCULAR BLOCKADE INDUCED BY D-TUBOCURARINE AND SUCCINYLCHOLINE (MEAN $\pm$ S.E., N = 4)

Percent Antagonism of d-Tubocurarine		
	Before	After
	Indomethacin	Indomethacin
PGE_2	6 $\pm$ 1	15 $\pm$ 3*
$PGF_{2\alpha}$	10 $\pm$ 1*	18 $\pm$ 2*
Percent Potentiation of Succinylcholine		
	Before	After
	Indomethacin	Indomethacin
PGE_2	5 $\pm$ 1	10 $\pm$ 2*
$PGF_{2\alpha}$	4 $\pm$ 1	12 $\pm$ 2*

*p < 0.05 when compared with control.

after indomethacin, but potentiated neuromuscular blockade by succinylcholine an average of four per cent (p > 0.05) before and 12 per cent (p < 0.05) after indomethacin (Table II).

DISCUSSION

Our results show that the cat sciatic-tibialis preparation responded in a dose-related manner to the administration of d-tubocurarine or succinylcholine and the neuromuscular blocking actions were not influenced by prostaglandin synthetase inhibition. "Prostaglandin synthetase" is a multi-enzyme complex, apparently present in every mammalian tissue thus far investigated.² "Aspirin-like drugs", although of diverse chemical structure, possess antipyretic, analgesic and anti-inflammatory activity in the decreasing order of potency: indomethacin > phenylbutazone > aspirin or ibuprofen.² Therefore, indomethacin was selected as the prototype of the prostaglandin synthetase inhibitors. Wilkerson and Glenn³ have previously demonstrated that, at the dosages used in this study, intravenous indomethacin 3 mg $\cdot$ kg⁻¹ intravenously will cause effective prostaglandin synthetase inhibition within 30 minutes of administration. Thus it seems that inhibition of prostaglandin biosynthesis alone does not alter the action of neuromuscular blocking agents.

However, the intra-arterial administration of exogenous prostaglandins PGE_2 and $PGF_{2\alpha}$ antagonized d-tubocurarine and potentiated succinylcholine neuromuscular blockade, although only the combination of $PGF_{2\alpha}$ with d-tubocurarine was statistically significant. These effects were greatly enhanced after indomethacin

administration. These results would be compatible with increased neurotransmitter release at the neuromuscular junction induced by these two prostaglandins.

PGE_1 and PGE_2 have been reported to reduce adrenergic nerve terminal release of norepinephrine in the isolated rabbit heart, while $PGF_{2\alpha}$ does not.¹⁻⁵ Jansson *et al.*⁶ have reported PGE_1 to inhibit acetylcholine release in the rat phrenic nerve-diaphragm preparation. It seems apparent, then, that different classes of prostaglandins in different species may increase or decrease neurotransmitter release at nerve terminals in which either acetylcholine or norepinephrine is the neurotransmitter.

Many of the prostaglandins, in particular the E series, influence the tissue levels of 3', 5'-AMP.^{7,8} It is currently thought that the E series prostaglandins activate membrane-bound adenylyl cyclase activity, especially in myocardium, by stimulation of a receptor site other than the beta-adrenergic receptor site; beta-blockade will not block this effect of the prostaglandins.⁸ Standaert, *et al.*¹⁰ recently demonstrated that activation of adenylyl cyclase, inhibition of phosphodiesterase and/or dibutyryl cAMP administration, increased motor nerve terminal 3', 5'-AMP levels and increased neurotransmitter output in the cat. Prostaglandin E_1 produced similar effects.¹⁰ Therefore, it appears that increases in nerve terminal 3', 5'-AMP induced pharmacologically may increase neurotransmitter release. These reports by other investigators are compatible with the data presented here. PGE_1 has been shown to have no effect on the response of skeletal muscle to acetylcholine,¹¹ further suggesting that the effects of the prostaglandins are on transmitter release at the nerve terminal.

Our data imply greater neurotransmitter release induced by PGE_2 and $PGF_{2\alpha}$ after prostaglandin synthetase inhibition, since an increase in acetylcholine concentration at the neuromuscular junction is expected to antagonize the non-depolarizing blockade of d-tubocurarine, but to potentiate the depolarizing blockade of succinylcholine. Samuelsson and Wennmalm¹² similarly observed that prostaglandin synthetase inhibition augmented release of norepinephrine from the isolated rabbit heart during sympathetic nerve stimulation, suggesting a prostaglandin-mediated control mechanism at the adrenergic nerve terminal. Prostaglandins may also modulate neurotransmitter release at the neuromuscular junction. It is possible that indomethacin may promote

acetylcholine release by inhibiting the synthesis of prostaglandins that normally limit neurotransmitter release.

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RÉSUMÉ

Les effets de l'inhibition de la biosynthèse des prostaglandines par l'indométhacine et les effets de l'injection artérielle de prostaglandines E₂ et F₂α avant et après l'inhibition de la biosynthèse des prostaglandines sur le blocage neuromusculaire obtenu par la d-tubocurarine et la succinylcholine ont été étudiés sur la préparation sciatique-tibiale du chat.

L'inhibition non spécifique de la biosynthèse prostaglandinique par l'indométhacine 3 mg · kg⁻¹ n'a pas modifié la période de latence, le sommet ou la durée du blocage neuromusculaire provoqué par la d-tubocurarine ou la succinylcholine.

La prostaglandine E₂ a antagonisé la dépression de l'amplitude de la secousse musculaire causée par la d-tubocurarine de 6 pour cent en moyenne avant et de 15 pour cent en moyenne après l'indométhacine, mais a potentialisé le bloc neuromusculaire de la succinylcholine par 5 pour cent en moyenne avant et par 60 pour cent en moyenne après l'indométhacine. La prostaglandine F₂α a antagonisé le blocage neuromusculaire de la d-tubocurarine par 10 pour cent en moyenne avant et par 18 pour cent après l'indométhacine, mais a potentialisé le blocage de la succinylcholine par 4 pour cent en moyenne avant et par 12 pour cent en moyenne après l'indométhacine.

Ces résultats semblent indiquer que l'inhibition non spécifique de la biosynthèse prostaglandinique seule n'a pas d'influence sur le blocage neuromusculaire provoqué par la d-tubocurarine et la succinylcholine. Cependant, les prostaglandines E₂ et F₂α peuvent provoquer une libération de médiateur à la jonction neuromusculaire possiblement accrue par l'indométhacine, ce qui aurait pour effet d'antagoniser le bloc non-dépolarisant de la d-tubocurarine et de potentialiser le bloc dépolarisant de la succinylcholine.