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LIGATED RENAL PEDICLES AND DURATION OF ACTION OF NEOSTIGMINE AND PYRIDOSTIGMINE

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1 The duration of neostigmine or pyridostigmine antagonism of a (+)-tubocurarine (Tc) neuromuscular blockade was determined in the cat anterior tibialis-peroneal nerve preparation with and without ligated renal pedicles.

2 The infusion rate of Tc required to maintain a 90% depression of twitch tension was reduced from 8.8 ± 1.4 (s.e.) to 3.4 ± 0.6 μg kg^{-1} min^{-1} by renal pedicle ligation.

3 Renal pedicle ligation resulted in an increased duration of antagonism of Tc by both neostigmine and pyridostigmine.

Introduction

Acetylcholinesterase inhibitors commonly are administered to antagonize the neuromuscular blockade produced by (+)-tubocurarine (Tc). The blockade from Tc rarely outlasts inhibition of acetylcholinesterase by neostigmine (Walts, Thorpe & Dillon, 1971). However, recent clinical reports suggest that the block may reappear when Tc presence is prolonged by the absence of renal function (Gibaldi, Levy & Hayton, 1972; Miller & Cullen, 1976). This implies that the absence of renal function does not equally influence the persistence of Tc and of neostigmine. In a separate study, the plasma half life of pyridostigmine, but not that of neostigmine, was prolonged in rats with ligated renal pedicles (Burdfield & Calvey, 1973). However, they did not correlate the plasma concentrations of neostigmine or pyridostigmine with inhibition of acetylcholinesterase or antagonism of Tc. Since pyridostigmine normally has a longer duration of action than neostigmine when antagonizing Tc (Miller, Van Nyhuis, Eger, Vitez & Way, 1974), the greater prolongation of pyridostigmine's presence by renal failure suggests it should be used, rather than neostigmine, to antagonize Tc during renal failure. We tested whether, indeed, absence of renal function differed in its effect on the durations of action of pyridostigmine and neostigmine when antagonizing a Tc neuromuscular blockade.

Methods

Thirty-one cats, 1.8 to 3.5 kg, were anaesthetized with chloralose, 60 mg/kg, and urethane, 500 mg/kg, intraperitoneally. Ventilation was controlled with a Harvard volume ventilator through a tracheostomy. The tendon of the anterior tibial muscle was freed, sectioned near its attachment, and connected to a Grass FT. 03 force-displacement transducer. The sciatic nerve was sectioned. The distal peroneal nerve was isolated and supramaximal stimuli (2 to 10 V) of 0.3 ms duration and 0.1 Hz were applied from a Grass stimulator (Model S4G) through shielded platinum electrodes to stimulate the muscle indirectly. The resulting force of muscle contraction was recorded continuously on a polygraph as discrete twitches in such a manner that twitch tension was proportional to isometric contractile force. In 17 of the 31 cats studied, the abdomen was opened, and both renal pedicles carefully identified and ligated.

A bolus intravenous injection of Tc, 0.2 mg/kg, then was given, after which Tc, 100 μg/ml, was infused from a Harvard pump at a rate that produced a constant 90% depression of twitch tension. The rate of infusion necessary decreased progressively for the next 15–45 min, after which it remained constant, as determined by at least 15 min of observation. Details of this constant-infusion technique have been described (Miller *et al.*, 1974). While the Tc infusion was continued at this rate, neostigmine or pyridostigmine was adminsitered as an intravenous bolus. Atropine, (10 μg/kg, i.v.) was given before neostigmine or pyridostigmine to prevent bradycardia and hypotension. The resultant maximum antagonism of twitch depression was calculated as a percentage of the pre-existing 90% depression (e.g., a peak rise to 40% of the pre-Tc twitch would be calculated as (40–10) 100/90, or 33% antagonism (Figure 1) (Miller *et al.*, 1974)). In addition, we measured times from neostigmine or pyridostigmine administration to peak effect (onset time) and to 50% return to the Tc-depressed twitch height (duration of action) (Figure 1). Subsequent doses of neostigmine or pyridostigmine

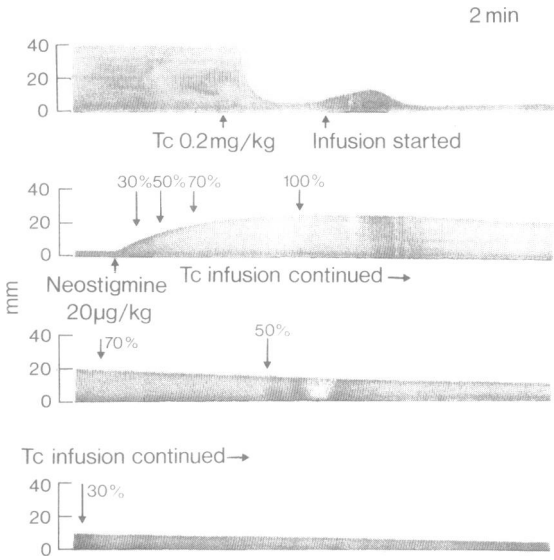

Figure 1 Example of one of the cat neuromuscular studies. After the infusion rate of (+)-tubocurarine (Tc) which produced constant 90% depression of twitch tension had been established, neostigmine was administered. Times from neostigmine administration to 30, 50, 70 and 100% of peak effect and 70, 50 and 30% to return of the Tc-depressed twitch can be determined.

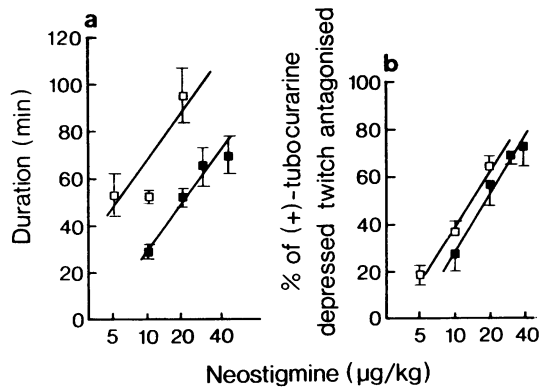

Figure 2 Correlation between dose of neostigmine (plotted on a log scale) and (a) duration of antagonism of the (+)-tubocurarine depressed twitch and (b) % of (+)-tubocurarine-depressed twitch antagonized. Duration was defined as time from neostigmine administration of 50% return to the (+)-tubocurarine depressed twitch tension. (■) Control; (□) ligated renal pedicles; each symbol represents the mean; vertical lines show s.e. mean. Ligation of renal pedicles significantly increased the duration of neostigmine action. The number of cats studied at each dose can be obtained from Figure 4.

were given after the twitch had returned to 90% depression for at least 15 minutes.

All the above values were compared in cats with and without ligation of renal pedicles which was performed before initiation of the Tc infusion. Arterial CO_2 tensions, pH, and rectal temperatures were maintained between 30–40 torr, 7.30–7.41, and 36–38°C respectively.

In four additional cats with ligated renal pedicles, Tc, 1.0 mg/kg, was administered as an intravenous bolus. Thirty minutes later neostigmine, 40 μg/kg, was administered intravenously. Twitch tension then was observed for 6 hours.

Analysis of covariance was used for part of the statistical analyses. Linear regression analysis and unpaired *t* tests were carried out for the remaining results.

Results

Ligation of renal pedicles did not alter twitch tension or arterial blood pressure. There was no difference in resting twitch tension between cats with and without ligated renal pedicles. However, ligation did reduce the infusion rates required to maintain a constant 90% depression of twitch tension from 8.9 ± 1.4 to 3.4 ± 0.6 μg kg^{-1} min^{-1} ($P < 0.01$). Accordingly, less neostigmine and pyridostigmine were required to antagonize the Tc depressed twitch during ligation of renal pedicles. Ligation of renal pedicles also reduced the ED_{50} of neostigmine and pyridostigmine (dose which resulted in a 50% antagonsim of Tc-depressed twitch) from 17 to 13.5 ($P < 0.05$) and from 145 to 78 μg/kg ($P < 0.01$) respectively (Figures 2 and 3). The neostigmine curves did not deviate from parallelism (Figure 2). Although the pyridostigmine curves appear to be nonparallel, their deviation is of marginal significance ($P < 0.10 > 0.05$) (Figure 3).

By analysis of variance or unpaired *t* test, the onset times were not affected by ligation of the renal pedicles (Figure 4) with one exception. By an unpaired *t* test the onset times with the 300 μg/kg dose of pyridostigmine were different (Figure 4) ($P < 0.01$). By analysis of covariance, the durations of action of both neostigmine and pyridostigmine were prolonged by ligation ($P < 0.01$) (Figures 2 and 3). Comparison of the individual doses was performed by an unpaired *t* test. The duration of action of 5 and 10 μg/kg doses of neostigmine in cats with ligated pedicles was longer than the 10 μg/kg dose in cats without ligation ($P < 0.01$) (Figure 2). The 20 μg/kg dose of neostigmine in cats with ligated pedicles had a longer duration of action than either the 20, 30, or 40 μg/kg dose in the cats without ligation ($P < 0.05$) (Figure 2). The 50 and 100 μg dose of pyridostigmine had a

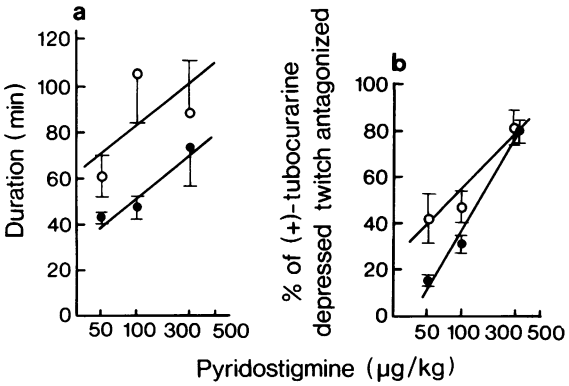

Figure 3 Correlation between dose of pyridostigmine (plotted on a log scale) and (a) duration of antagonism of the (+)-tubocurarine depressed twitch tension and (b) the % of (+)-tubocurarine depressed twitch antagonized. Duration was defined as time from pyridostigmine administration to 50% return to the (+)-tubocurarine depressed twitch tension. Ligation of renal pedicle significantly increased the duration of pyridostigmine action. (●) Control; (○) ligated renal pedicles; each symbol represents the mean; vertical lines show s.e. mean. The number of cats studied at each dose can be obtained from Figure 4.

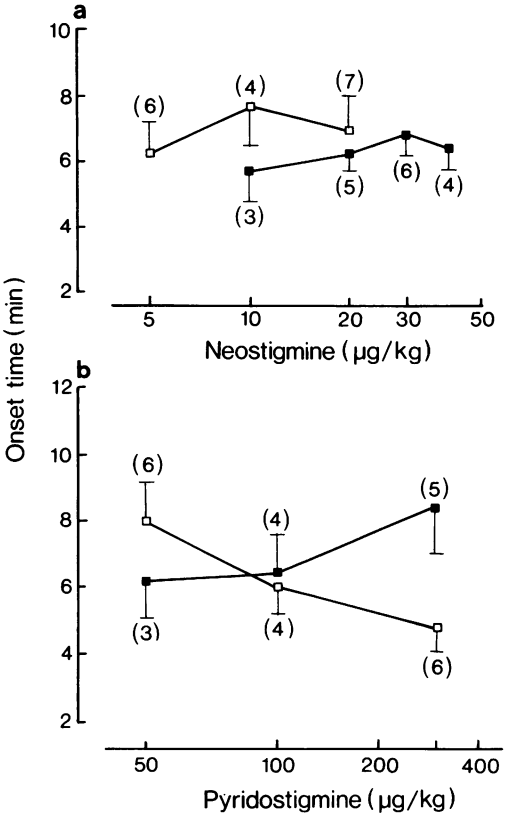

Figure 4 Correlation between (a) dose of neostigmine or (b) pyridostigmine and time to peak antagonism. (■) Control; (□) ligated renal pedicles; each symbol represents the mean; vertical lines show s.e. mean. The numbers in parentheses represent the number of cats studied at each dose.

longer duration of action in cats with ligation than those without ligation ($P<0.10$ and $P<0.01$ respectively, Figure 3). Although the duration of action of 300 μg/kg of pyridostigmine was longer in cats with ligation these differences were not significant (Figure 3).

A 100% depression of twitch tension followed the intravenous administration of Tc, 1.0 mg/kg in four cats. Thirty minutes later, at which time the twitch was still depressed 100%, administration of neostigmine, 40 μg/kg intravenously resulted in a mean 84% antagonism of the Tc blockade. In the next 6 h twitch tension did not decrease. In fact it gradually continued to increase to the control (pre Tc) tension.

Discussion

Our results indicate that ligation of renal pedicles prolongs the effect of both neostigmine and pyridostigmine. Yet Burdfield & Calvey (1973) found the plasma half-life of neostigmine was not prolonged by renal pedicle ligation in rats. The difference from our results may be explained by the difference in the measured variable (twitch tension and antagonism of Tc versus drug concentration in plasma). In addition to a difference in species, the problems of equating intensity of pharmacological action with plasma concentrations of drugs are well known (Koch-Weser, 1972). The importance of renal excretion to the elimination of both neostigmine and pyridostigmine is suggested by the fact that 40–60% of both neostigmine and pyridostigmine are excreted in the urine (Nowell, Scott & Wilson, 1962; Roberts, Thomas & Wilson, 1965; Birtley, Roberts, Thomas & Wilson 1966). Our results of prolonged duration of action of both drugs therefore are not surprising.

We believe the reduced infusion rate of Tc required for 90% depression of twitch tension and prolonged duration of neostigmine and pyridostigmine action during ligation of renal pedicles are entirely due to absence of renal function. That arterial blood pressure did not change by ligation of renal pedicles suggests that significant amounts of vasoactive substances were not released. Resting twitch tension did not change when the pedicles were ligated which suggests neuromuscular transmission was not altered. Although anaesthetic levels may have been different in

the two groups of cats, chloralose has little or no effect at the cat neuromuscular junction (Hoekman, Dretchen & Standoert, 1974).

The duration of action of neuromuscular blocking drugs, Tc and pancuronium, and their antagonists, neostigmine and pyridostigmine, probably are prolonged to a similar degree by the absence of renal function (Gibaldi *et al.*, 1972; Miller, Stevens & Way, 1973; McLeod, Watson & Rawlins, 1976). This conclusion should be guarded since the duration of action of these drugs has not been studied well in the cat or man in the absence of renal function. If true, however, these results suggest that blockade from Tc should not outlast inhibition of acetylcholinesterase by neostigmine. We further tested this suggestion by administering a dose of Tc 3.5 times that required to depress twitch tension by 100% in cats with ligated renal pedicles. Subsequent administration of neostigmine resulted in a prompt and lasting antagonism of Tc. However, studies in which the pharmacokinetics or sensitivity of the neuromuscular junction to these drugs is changed by altered physiological states are required before one can assume that the blockade from TC will not outlast inhibition of acetylcholinesterase by neostigmine.

In spite of our results to the contrary, clinical reports suggest that a neuromuscular blockade may reappear when the presence Tc is prolonged by absence of renal function (Miller & Cullen, 1976; Gibaldi *et al.*, 1972) which suggests that renal excretion is more important to the termination of Tc than neostigmine action. These reports may be explained by concomitant administration of diuretics (Miller, Sohn & Matteo, 1976) or antibiotics (Pittinger & Adamson, 1972) both of which commonly are given to patients with renal failure and may augment a Tc neuromuscular blockade. Alternatively, perhaps the doses of Tc were too large and doses of neostigmine too small. These questions cannot be answered until Tc, neostigmine, and pyridostigmine concentrations and/or effects are measured in patients with and without renal failure. In any event, our results in cats indicate that the durations of neostigmine and pyridostigmine antagonism of a Tc blockade are both prolonged by renal pedicle ligation. These results suggest that the blockade from Tc should not outlast inhibition of acetylcholinesterase by neostigmine in patients with absence of renal function.

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References

BIRTLEY, R.D.N., ROBERTS, J.B., THOMAS, B.H. & WILSON, A. (1966). Excretion and metabolism of [^{14}C]-pyridostigmine in the rats. *Br. J. Pharmac.*, **26**, 393–402.

BURDFIELD, P.A. & CALVEY, T.N. (1973). Plasma clearance of neostigmine and pyridostigmine in rats with ligated renal pedicles. *Eur. J. Pharmac.*, **24**, 252–255.

GIBALDI, M., LEVY, G. & HAYTON, W.L. (1972). Tubocurarine and renal failure. *Br. J. Anaesth.*, **44**, 163–165.

HOEKMAN, T.B., DRETCHEN, K.L. & STANDAERT, F.G. (1974). Miniature end plate potentials recorded from mammalian myoneural junction in vivo. *Science*, **183**, 213–215.

KOCH-WESER, J. (1972). Serum drug concentrations as therapeutic guides. *New Eng. J. Med.*, **287**, 227–230.

McLEOD, K., WATSON, M.J. & RAWLINS, M.D. (1976). Pharmacokinetics of pancuronium in patients with normal and impaired renal function. *Br. J. Anaesth.*, **48**, 341–345.

MILLER, R.D., VAN NYHUIS, L.S., EGER, E.I., II, VITEZ, T.S. & WAY, W.L. (1974). Comparative times to peak effect and durations of action of neostigmine and pyridostigmine. *Anesthesiology*, **41**, 27–33.

MILLER, R.D. & CULLEN, D.J. (1976). Renal failure and postoperative respiratory failure: recurarization? *Br. J. Anaesth.*, **48**, 253–256.

MILLER, R.D., SOHN, Y.J. & MATTEO, R.S. (1976). Enhancement of d-tubocurarine neuromuscular blockade by diuretics in man. *Anesthesiology*, **45**, 442–445.

MILLER, R.D., STEVENS, W.C. & WAY, W.L. (1973). The effect of renal failure and hyperkalemia on the duration of pancuronium neuromuscular blockade in man. *Anesth. Analg.*, **52**, 661–666.

NOWELL, P.T., SCOTT, C.A. & WILSON, A. (1962). Determination of neostigmine and pyridostigmine in the urine of patients with myasthenia gravis. *Br. J. Pharmac. Chemother.*, **18**, 617–624.

PITTINGER, C.B. & ADAMSON, R. (1972). Antibiotic blockade of neuromuscular function. *Ann. Rev. Pharmac.*, **12**, 169–184.

ROBERTS, J.B., THOMAS, B.H. & WILSON, A. (1965). Distribution and excretion of [^{14}C]-neostigmine in the rat and hen. *Br. J. Pharmac.*, **25**, 234–242.

WALTS, L.F., THORPE, W.K. & DILLON, J.B. (1971). Recurarization—fact or fiction. *Anesth. Analg.*, **50**, 879–885.

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