ESERINE AND NEOSTIGMINE ANTAGONISM TO D-TUBOCURARINE

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THE use of curare as an adjuvant in anesthesia increases interest in curare antidotes. Recently, McIntyre, Tullar and Frank (1) reported that a mixture of eserine, ephedrine and atropine was superior to neostigmine in counteracting the action of d-tubocurarine in dogs. Subsequently Burke and Linegar at the Squibb Laboratories were unable to confirm these observations, and it was decided that this question be jointly re-examined and an explanation sought for the difference in experimental findings. It was discovered in the course of these experiments that the cause of the discrepancy lay in the sample of neostigmine previously used by McIntyre et al. It was determined that this sample supplied by another manufacturer as pure prostigmin bromide was, in fact, a different compound under investigation by them; i.e., a primary amine containing a nitrogenous ring and subsequently shown to produce hypotensive responses in small doses.

This paper presents data obtained from experiments performed for the most part in duplicate by the two laboratories on the relative value of neostigmine methyl sulfate and either eserine salicylate alone or eserine with atropine sulfate and ephedrine sulfate as antidotes to d-tubocurarine chloride.

PROCEDURE

The experiments fall into two categories, acute experiments on anesthetized dogs and experiments on intact unanesthetized animals. In the former, animals were anesthetized with either morphine and sodium barbital in the technic of Wiggers (2) or by cyclopropane-oxygen administered by a closed system. No measurable differences in results were observed which could be attributed to the anesthetic.

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The anesthetized animals were arranged for the kymographic recording of muscle contractions when the muscles were driven by either monophasic spike shocks applied to the severed sciatic at five second intervals or by short bursts of tetanic stimuli usually applied at ten second intervals. Blood pressure and respiration were also recorded in many of the experiments.

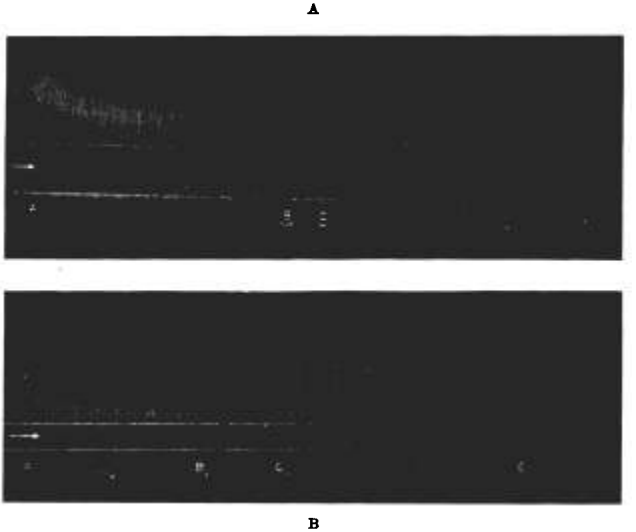

FIG. 1A. Monophasic spike shocks to cut sciatic nerve at five second intervals.

A. d-tubocurarine chloride 0.18 mg./kg. intravenously.
B. d-tubocurarine chloride 0.18 mg./kg. intravenously.
C. 2 cc. of 0.1% solution of eserine salicylate intravenously.
D. Same as A.
E. Same as A.

FIG. 1B. Monophasic spike shocks to cut sciatic at five second intervals.

A. d-tubocurarine chloride 0.18 mg./kg. intravenously.
B. d-tubocurarine chloride 0.18 mg./kg. intravenously.
C. 2 cc. of 0.05% solution of Neostigmine methyl sulfate intravenously.
D. Same as A.
E. Same as A.

In the experiments on unanesthetized animals, the head-drop dose of d-tubocurarine chloride was determined by several trials on each of the experimental animals and the duration of head-drop noted. In some experiments the dosage of curare was increased to 120, 140, 160 or 300 per cent of the head-drop dose. Injections were repeated with

neostigmine, eserine with atropine, or eserine with atropine and ephedrine administered immediately after the curare as antidotes and the duration of curarization compared with that obtained without antidotes.

EXPERIMENTAL RESULTS

Figure 1 depicts the records obtained in two typical experiments upon anesthetized dogs in which the muscles were driven by single

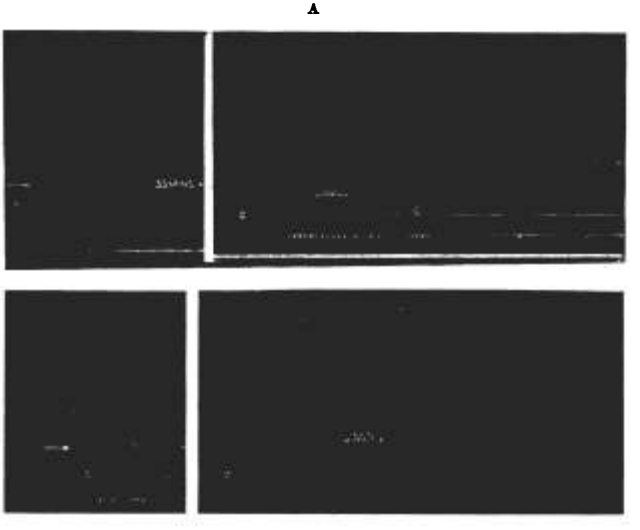

FIG. 2A. Monophasic tetanizing shocks applied to cut sciatic.

A. d-tubocurarine solution injected slowly until tetanic contraction of muscle was not sustained. Tetanic response returned after approximately 35 minutes.
B. d-tubocurarine solution injected as in A.
C. Eserine salicylate 0.125 mg./kg. intravenously.

FIG. 2B. Monophasic tetanizing shocks applied to cut sciatic.

A. d-tubocurarine solution injected slowly until tetanic contraction of muscle was not sustained. Tetanic response returned after approximately 15 minutes.
B. d-tubocurarine solution injected as in A.
C. Neostigmine methyl sulfate 0.125 mg./kg. intravenously.

spike shocks applied to the cut sciatic and the effects of d-tubocurarine alone and as modified by eserine or by neostigmine were compared. Figure 2 contains the records obtained from two experiments of the series in which bursts of tetanic stimulation were applied at regular

TABLE 1

COMPARATIVE RECOVERY TIME OF DOGS FOLLOWING VARIOUS DOSES OF D-TUBOCURARINE CHLORIDE WITH AND WITHOUT NEOSTIGMINE OR ESERINE

Wt. in kg.	Initial standardization			Curare dose: 120% of H.D. 2 u./cc. (0.5 cc./10 sec.)			Curare dose: 140% of H.D. 2 u./cc. (0.5 cc./10 sec.)			Curare dose: 160% of H.D. 2 u./cc. (0.5 cc./10 sec.)		
	Average H. D. dose		Average recovery time in min.	Recovery time, no antidote	Recovery time with eserine (0.125 mg./u. curare) and atropine (0.1 mg./kg.)	Recovery time with neostigmine (0.05 mg./u. curare)	Recovery time, no antidote	Recovery time with eserine (0.125 mg./u. curare) and atropine (0.1 mg./u. curare)	Recovery time with neostigmine (0.05 mg./u. curare)	Recovery time, no antidote	Recovery time with eserine (0.125 mg./u. curare) and atropine (0.1 mg./u. curare)	Recovery time with neostigmine (0.05 mg./u. curare)
	Total units §	Units per kg.										
kg.	units	u./kg.	min.	min.	min.	min.	min.	min.	min.	min.	min.	min.
10.0	8.8	0.88	10.83	20.17	3.00	1.00	20.33	3.75	1.35	43.17	7.25	2.08
14.6	12.2	0.83	5.02	9.72	2.33	0.33	9.25	2.83	3.58	20.00	4.25	2.25
16.9	17.2	1.02	4.46	5.50	0.67	0.50	12.58	2.00	0.75	20.00	4.33	2.00
16.3	15.8	0.97	4.86	7.67	0.67	0.63	11.75	2.17	1.25	29.72	7.25	2.33
22.2	12.8	0.58	7.17	5.67	2.00	1.00	18.08	4.67	0.92	18.74	3.75	2.50
14.6	16.0	1.09	5.12	7.25	2.55	0.70	22.25	3.33	1.12	44.13	3.58	1.50
*13.9	12.2	0.88	4.33	8.75	1.92	0.50	25.00	4.33	2.88			
†26.8	34.0	1.27	7.78	17.00	3.50	1.25						
‡15.0	17.0	1.13	10.75		5.08	0.25						
Average		0.961 u./kg.	6.80	10.22	2.41	0.68	17.03	3.30	1.69	30.79	5.07	2.11
			S.E.:	±18.75%	±19.05%	±16.38%	±14.38%	±11.77%	±24.33%	±14.49%	±13.81%	±6.73%

* Died within 24 hours after receiving curare 140 per cent of H.D. and no antidote.

† Received curare 140 per cent of H.D. and no antidote—resp. par. in 8 min.—1 cc. neostigmine (I.V.), 8.8 min. 1 cc. neostigmine (I.V.), dead 12.5 min.

‡ Received curare 120 per cent of H.D. and neostigmine—died within ½ hour after apparent recovery.

§ 1 unit = 0.154 mg. d-tubocurarine chloride pentahydrate.

intervals to the cut sciatic. The slow intravenous injection of a solution of d-tubocurarine causes a rapid loss of tetanic contraction. It will be observed in figure 1 that neostigmine caused a more abrupt recovery than eserine. In figure 2 it will be seen that neostigmine caused a greater degree of recovery of tetanic contractions than did eserine.

TABLE 2

AVERAGE DURATION OF CURARE HEAD-DROP IN DOGS WITH AND WITHOUT ANTIDOTE

Dog No.	d-tubocurarine 0.18 mg.*/kg.	d-tubocurarine 0.18 mg./kg. with eserine, atropine, ephedrine
	min.	min.
1	9.83	7.34
2	12.16	5.91
3	4.33	4.25
4	7.16	5.41
5	5.75	3.83
6	7.66	5.54

* 1 mg. d-tubocurarine chloride pentahydrate = 6.5 units.

TABLE 3

DURATION OF RESPIRATORY PARALYSIS, HEAD-DROP AND ATAXIA IN DOGS GIVEN THREE HEAD-DROP DOSES OF D-TUBOCURARINE CHLORIDE WITH AND WITHOUT NEOSTIGMINE

Dog No.	Wt. in kg.	Curare H.D. dose, units*	Total dose of curare administered, units	d-Tubocurarine chloride only			d-Tubocurarine chloride (3 head-drop doses) + neostigmine (0.0167 mg./u. of curare) immediately following curare injection			d-Tubocurarine chloride (3 head-drop doses) + neostigmine (0.0167 mg./u. of curare) 15 minutes after curare injection		
				Respiratory paralysis	Head-drop	Ataxia	Respiratory paralysis	Head-drop	Ataxia	Respiratory paralysis	Head-drop	Ataxia
				Time in minutes from respiratory failure			Time in minutes from neostigmine			Time in minutes from neostigmine		
2877	9.5	6.0	18.0	57.42	108.33	130.17	4.92	51.17	76.00	20.75	39.75	48.75
1714	13.5	6.0	18.0	53.00	53.00	78.00	1.00	30.92	30.92	2.75	26.92	30.25
2852	10.9	5.5	16.5	29.75	51.75	66.75	10.00	43.50	43.50	1.67	5.67	37.67
1712	16.4	6.0	18.0	25.58	46.58	48.00	14.00	17.50	17.50	4.33	21.50	26.08
2842	10.2	5.5	16.5	51.00	55.50	61.25	6.82	20.08	20.08	1.75	8.25	19.50
Mean (log) ratio of Duration with neostigmine / Dur. without neostigmine ± S.E.(%):							.1289± 61.2%	.5006± 14.7%	.4481± 14.2%	.1436± 17.8%	.2691± 31.6%	.4273± 10.3%

* 1 unit = 0.154 mg. d-tubocurarine chloride pentahydrate.

Table 1 presents data obtained from a representative series of animals in which the relative effectiveness of eserine with atropine and neostigmine as antidotes to d-tubocurarine were compared at increasing dosage levels of curare. It will be seen that neostigmine proved superior to eserine. In another series of animals the effectiveness of

a mixture of ephedrine, atropine and eserine was determined. Although this mixture significantly shortened the duration of head-drop, it was less effective than neostigmine (table 2). A third group of dogs given 3 head-drop doses of d-tubocurarine required artificial respiration for various periods of time. Neostigmine 0.0167 mg. per unit (0.154 mg) of curare given either immediately or fifteen minutes after the curare significantly reduced the recovery time from curarization (table 3). Greater total dosage of neostigmine was not attempted for fear of blood pressure depression.

DISCUSSION

In curare overdosage, the most effective treatment is prompt intratracheal intubation and the application of artificial respiration with oxygen.* This is perhaps best accomplished by the rhythmic application of pressure to the anesthetic bag. In dogs it has been found that intubation should be performed routinely because very frequently in fully curarized animals the relaxed muscles may result in obstruction of the respiratory passages. Artificial respiration alone when performed as outlined above will usually afford sufficient support until spontaneous respiration occurs. The use of neostigmine will shorten the period for the necessity of artificial respiration, but its administration should be considered as an adjuvant to treatment rather than as a substitute.

Since 1900 when Pal (3) reported upon eserine-curare antagonism and the subsequent synthesis of neostigmine and other methylcarbamic esters, the use of these substances as curare antidotes has become well established. In 1922 Hori (4) attempted to place eserine-curare antagonism on a quantitative basis but concluded that the antagonism is limited. Large doses of eserine may result in a loss of indirect excitability. Koppanyi and Vivino (5) found that eserine in doses of 0.1 mg./kg. in rabbits prevented death in animals that had received 1.5 units/kg. of d-tubocurarine. The same authors found that neostigmine was equally effective in about one-half the dose. These results agree with those reported above. It should be pointed out that the dosage of neostigmine found effective in experimental animals is considerably greater than that commonly recommended for clinical use in man. It appears likely that the recommended clinical dose of 1.0 cc. of 1:2,000 neostigmine methylsulfate is too low to be effective and it is suggested that for a 75 kg. patient from three to five times this amount be used.

CONCLUSIONS

1. In d-tubocurarine overdosage the intravenous injection of neostigmine in dogs promptly restores indirect excitability of muscle and restores the ability of the muscle to maintain tetanic contractions.

2. Neostigmine is superior to eserine and eserine-atropine-ephedrine mixtures as an antidote to d-tubocurarine.

3. The dosage of neostigmine heretofore used clinically in curare overdosage is probably inadequate.

* O_2CO_2 mixtures should not be used.

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250th Station Hospital	Regensburg, Germany	150
279th Station Hospital	Berlin, Germany	350
317th Station Hospital	Wersbaden, Germany	150
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