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Passage of intravenously administered tubocurarine into the liquor space in man and dog

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Summary

1. In anaesthetized patients under controlled respiration, samples of lumbar cerebrospinal fluid were withdrawn 15 and 60 min after an intravenous injection of 30 mg tubocurarine. When tested on the frog rectus muscle preparation contracted by acetylcholine, they exerted curare-like activity which corresponded to between 0·05 and 0·33 μg/ml tubocurarine.

2. In dogs anaesthetized with pentobarbitone sodium and artificially ventilated, two procedures were adopted to find out if tubocurarine passes into the liquor space after an intravenous injection of 0·3 or 3 mg/kg and during its intravenous infusion at a rate of 10 (μg/kg)/minute. Either samples of cisternal cerebrospinal fluid (c.s.f.) were collected, or different regions of the liquor space were perfused with artificial c.s.f. and the effluent was collected. The samples of c.s.f. and the effluent were assayed for curare-like activity on the frog rectus muscle.

3. After the intravenous injection of tubocurarine samples of cisternal effluent collected during perfusion from lateral ventricle to cisterna exerted curare-like activity. It corresponded to 20 ng/min tubocurarine in the sample collected during the first 15 min after the injection of 0·3 mg/kg and to 40–60 ng/min in the samples collected up to 2 h after the injection of 3 mg/kg.

4 During intravenous infusion of tubocurarine the cisternal c.s.f. as well as the effluent from the perfused regions of the liquor space exhibited curare-like activity. Expressed in equivalents of tubocurarine, the activity in the cisternal c.s.f. ranged from between 0·1 and 0·75 μg/ml. On perfusion from lateral ventricle to aqueduct or cisterna, the activity ranged from between 3 and 25 ng/min in the aqueductal and from between 4 and 40 ng/min in the cisternal effluent. On perfusion from the lumbar-spinal subarachnoid space to cisterna it ranged from between 6 and 55 ng/min in the cisternal effluent.

Introduction

In clinical anaesthetic practice, tubocurarine is administered to obtain muscle relaxation, frequently in doses that produce paralysis of respiratory muscles, and the patients are given controlled respiration. Gray & Rees (1952) advocated controlled respiration after inducing apnoea with muscle relaxants as a desirable anaesthetic procedure. This procedure has received wide clinical application. Tubocurarine 8–10 mg (0·1–0·2 mg/kg) given i.v. to anaesthetized patients produces signs of neuromuscular block which spares the respiratory muscles; but with 15–20 mg (0·3–0·4 mg/kg) respiratory paralysis occurs as well (Wylie & Churchill-Davidson, 1960).

Other authors (Kovanev & Khmelevsky, 1964; Boutros, 1965; Neill & Nixon, 1965; Nightingale & Richards, 1965) have used even larger doses, between 25 and 45 mg (0·4–0·9 mg/kg) and in the General Hospital of Kurnool Medical College, 30 mg tubocurarine is given routinely in general anaesthesia to produce muscle relaxation under controlled respiration.

To find out if under these conditions tubocurarine appears in the cerebrospinal fluid (c.s.f.) samples of lumbar c.s.f. were collected from anaesthetized patients after the tubocurarine injection and assayed for tubocurarine activity by the bio-assay method of Burn (1952). With this method, small amounts of tubocurarine can be detected by their ability to relax the frog-rectus muscle when contracted by acetylcholine. Additional experiments were carried out on anaesthetized dogs in which tubocurarine was injected or infused intravenously. In these experiments the tubocurarine assay was carried out either on c.s.f. collected from the cisterna magna or on effluent collected during perfusion of different parts of the liquor space with artificial c.s.f. Plasma levels of tubocurarine were determined as well in samples of arterial blood collected during the intravenous tubocurarine infusion.

Earlier reports on the passage of tubocurarine into c.s.f. are conflicting. Mahfouz (1949) found, in a patient, that c.s.f. taken after an intravenous injection of tubocurarine (0·2 mg/kg) exerted curare-like activity equivalent to 2·5 μg/ml when tested on the frog rectus muscle In anaesthetized dogs, Dal Santo (1964) found that after an intravenous injection of a trace dose of ^{14}C-dimethyl-(+)-tubocurarine, equivalent to 25×10^6 counts per min, up to 20×10^{-5} of the injected amount could be detected in the cisternal c.s.f. On the other hand, Cohen (1963) failed to detect any tubocurarine with chemical methods of assay in c.s.f. of dogs anaesthetized with pentobarbitone sodium which had received intravenous or intracarotid injection of 0·3–3 mg/kg tubocurarine, although tubocurarine was detected in the plasma with his methods after these injections. He was also unable to detect with his chemical methods any tubocurarine in c.s.f. samples collected from human volunteers under thiopentobarbitone anaesthesia during a period of 1 h after an intravenous injection of 0·3 to 0·6 mg/kg tubocurarine.

Methods

Clinical procedures

Six patients (44 to 70 kg) were given, after pre-medication with atropine (0·6 mg) an intravenous injection of 30 mg tubocurarine followed by 350 mg thiopentone. The injections produced immediate respiratory paralysis. The trachea was therefore at once intubated, connexion was established with a Boyle's apparatus, and controlled respiration was applied. Anaesthesia was maintained with nitrous oxide 70% and oxygen 30%. Three samples of c.s.f. were collected, the first before, the second 15, and the third 60 min after the tubocurarine injection. Surgical procedures were begun after the second sample was taken.

Experimental procedures

Dogs weighing 6·5 to 19 kg were used. They were anaesthetized with intravenous pentobarbitone sodium (30 mg/kg). The trachea was cannulated and respiration was recorded by means of a tambour. Blood pressure was recorded with a mercury manometer from the cannulated left femoral artery. The right femoral vein was

cannulated with a polyethylene tube for administration of tubocurarine. It was either given as a single injection or infused at a rate of 0·4 ml/min with a slow injector. Artificial ventilation was applied with an 'ideal' respiratory pump, immediately after the intravenous injection, or when the expiratory phase in the respiratory record diminished during the intravenous infusion.

Collection of blood samples

Blood samples were taken from the right femoral artery, placed in centrifuge tubes containing 0·1 ml heparin and immediately centrifuged at 2,000 r.p.m. for 15 minutes. The plasma was separated and stored in the refrigerator for assay.

Collection of c.s.f.

With the dog lying on its belly, the head was fixed to the ear bars and mouthpiece of a stereotaxic instrument. The atlanto-occipital membrane was exposed by dissection of the muscles at the back of the neck and the cisterna magna was punctured by a needle (gauge 18). A polyethylene tube was attached to the free end of the needle which was held in position by a clamp. The polyethylene tube was closed by a stopper which was removed each time a sample of c.s.f. (about 1 ml) was withdrawn.

Perfusion of the liquor spaces

Perfusion was either from a Collison cannula implanted into the left lateral ventricle to cisterna magna or aqueduct, or from a polyethylene tube introduced into the lumbar spinal subarachnoid space to cisterna magna. The methods were the same as those described for cats by Bhattacharya & Feldberg (1958a) for perfusion from the lateral ventricle and by Haranath, Premalatha & Sunanda-bai (1966) for perfusion from the spinal subarachnoid space. The Collison cannula used was that originally described by Feldberg & Sherwood (1953) as modified by McCarthy & Borison (1966).

In some experiments in which the perfusion was from the lateral ventricle the outflow was collected first from the cisterna magna and later from the aqueduct. When perfusion was from the spinal subarachnoid space to cisterna magna it was changed in some experiments after the first 1·5 h; perfusion was then continued from the lateral ventricle which had been cannulated at the beginning of the experiment to the cisterna and later from the lateral ventricle to the aqueduct.

The fluid used for the perfusions was the artificial c.s.f. of Merlis (1940). Its composition was (g/l.): NaCl 8·1; KCl 0·25; $CaCl_2$ 0·14; $MgCl_2$ 0·11; $NaHCO_3$ 1·76; NaH_2PO_4 0·07; urea 0·13; glucose 0·61. Perfusion was at a rate of 0·1 ml/min with a continuous slow injector. The effluent was collected in graduated tubes and stored at 0–4° C until assayed. If the effluent was blood-stained, the experiment was discarded.

Tubocurarine assay

The method of Burn (1952) for the assay of curare-like activity on the acetylcholine contractions of the frog rectus muscle was used, but it was made more sensitive by the following modifications. The size of the bath was reduced to

1 ml. Minimal amounts of acetylcholine (0·05 to 0·15 μg) were used to contract the muscle which was rendered sensitive to acetylcholine by the addition of physostigmine (10 μg/ml) to the bath fluid. The samples to be assayed were kept in contact with the muscle for 2 min before applying the acetylcholine, instead of the 30 s contact recommended by Burn. With these modifications definite depression of contractions to acetylcholine was obtained with 0·05 to 0·1 μg tubocurarine. The samples of plasma, c.s.f. and perfusate were assayed either on the day of collection or after storage overnight at 0–4° C.

In preliminary experiments it was found that c.s.f. and perfusate did not affect the assay but when tubocurarine was assayed in plasma the values were only about 50% of the tubocurarine content. The pronounced potentiating effect which plasma exerts on the acetylcholine contractions probably accounts for this effect, as it counteracts the depressant effect of tubocurarine. The tubocurarine was (+)-tubocurarine chloride (Koch-Light, England). It was dissolved in 0·9% w/v NaCl solution. All values refer to its salt.

Results

Clinical studies

Control samples of c.s.f. collected from the six patients did not show any tubocurarine effect, but all samples collected 15 min after the intravenous injection of 30 mg tubocurarine exerted a curare-like effect equivalent to between 0·05 and 0·33 μg/ml. The effect on the frog rectus muscle is shown for one experiment in Figure 1. The control sample added to the bath in a volume of 0·4 ml did not affect

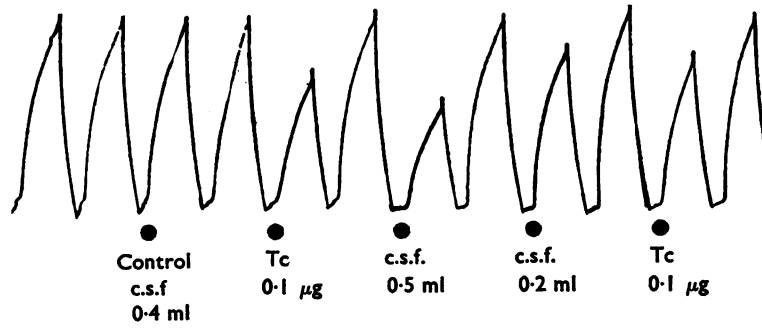

FIG. 1. Contractions of the frog rectus muscle suspended in 1 ml bath to 0·1 μg acetylcholine. The black dots indicate that 2 min before the addition of ACh the following additions were made to the bath: at the first dot 0·4 ml human c.s.f. collected before, at the third and fourth dots 0·5 and 0·2 ml collected after an intravenous injection of 30 mg tubocurarine; at the second and fifth dots 0·1 μg tubocurarine (Tc). Same experiment as experiment 1 in Table 1.

the acetylcholine contraction but 0·5 and even 0·2 ml of the sample collected 15 min after the tubocurarine injection caused a depression. The curare effect of this sample corresponded to 0·33 μg/ml tubocurarine. The results obtained from all six patients are summarized in Table 1 which also gives the tubocurarine equivalents of the samples of c.s.f. collected 60 min after the injection. These samples still exerted curare-like activity although it was less than that of the 15 min samples.

The intravenous injections of tubocurarine produced on the arterial blood pressure a transient fall during the first 5 min followed by a rise above the pre-injection

TABLE 1. *Curare-like effect (expressed in terms of tubocurarine μg/ml) in c.s.f. collected from six patients 15 and 60 min after 30 mg i.v. tubocurarine (Tc)*

Patient			Tc (μg/ml)	
No.	Sex	Weight in kg	15 min	60 min
1.	Male	50·0	0·33	0·1
2.	Male	52·3	0·2	0·09
3.	Male	70·0	0·17	<0·05
4.	Male	45·0	0·2	
5.	Male	45·4	0·05	<0·05
6.	Female	44·0	0·25	

level. The results obtained from the first four patients of Table 1 are given graphically in Figure 2. In three of the patients, blood pressure returned to the pre-injection level within 20 to 30 min, in the fourth it took about 60 minutes.

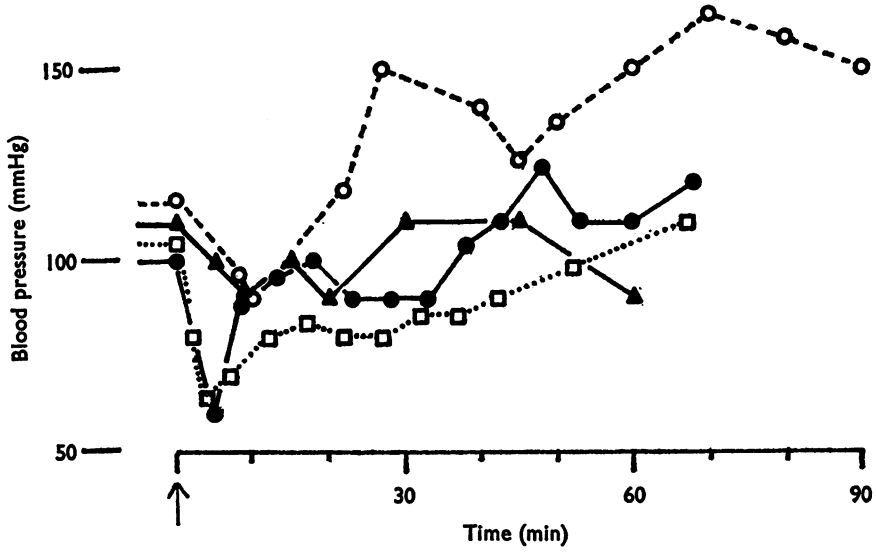

FIG. 2. Blood pressure changes during controlled respiration in four patients after tubocurarine 30 mg and thiopentone 350 mg given i.v. at the arrow. Same patients as in Table 1. Patient 1 (●); 2 (▲); 3 (○); 4 (□).

Experiments on dogs

Intravenous infusion of tubocurarine

Effects on respiration and arterial blood pressure

The intravenous infusion of 10 (μg/kg)/min tubocurarine into anaesthetized dogs resulted after a latency of 15–45 min in slight respiratory depression. The rate of respiration increased as the respiratory excursion diminished. Depression slowly progressed and when the expiratory phase was completely depressed, artificial ventilation was begun. In most experiments the initial blood pressure was between 140 and 160 mmHg and did not change until respiration became depressed; it then fell by about 10 to 40 mmHg before artificial respiration was begun. After this it showed mild fluctuations but otherwise remained unchanged.

Curare-like activity in plasma

Plasma samples obtained at various intervals during a continuous intravenous infusion of tubocurarine for 4 h showed a curare-like effect on the frog rectus muscle if the rate of infusion was 10 (μg/kg)/min, but not at a rate of 1 (μg/kg)/minute. Table 2 summarizes the results of 15 experiments in which the rate of infusion was 10 (μg/kg)/minute. The first sample collected 30 min after the onset of infusion exhibited some curare-like activity which increased steadily in the subsequent samples. The increase is particularly evident from the mean values given at the bottom of the Table. The experiments are arranged in order of increasing weight of the dogs, and although some of the highest plasma concentrations were obtained from the largest dogs, in which the absolute amounts of tubocurarine infused were naturally greatest, there was no clear-cut dependence on weight.

TABLE 2. *Concentration of tubocurarine in consecutive half hourly or hourly samples of plasma obtained from dogs during a continuous intravenous infusion of* 10 (μg/kg)/min *tubocurarine (Tc)*

Experiment No.	Weight of dog in kg	Tc (μg/ml) 0·5 h	1 h	2 h	3 h	4 h
1	6·5		0·14	0·13	0·20	0·25
2	7·5	0·17	0·25	0·59	0·66	
3	8·2		0·50	0·77	1·00	
4	8·4	0·18	0·38	0·50		
5	9·0		0·33	0·58	1·00	
6	9·0		0·29	0·57	0·67	
7	9·5		0·33	0·67	1·34	
8	9·5		0·83	0·50	0·66	
9	11·0		0·33	0·33	1·00	4·00
10	11·5	0·08	0·08	0·25	0·14	
11	11·7		0·33	0·83	1·43	
12	12·0		0·38	0·45	0·43	0·50
13	14·5		0·85	2·00	0·30	
14	14·5		Nil	0·50	0·83	1·00
15	19·0	0·66	1·00			
	Mean	0·26	0·36	0·58	0·71	1·19

Curare-like activity in cisternal c.s.f.

No curare-like effect was obtained in c.s.f. samples collected during intravenous infusion at a rate of 1 (μg/kg)/min tubocurarine. On infusion at a rate of 5 (μg/kg)/min a curare-like effect equivalent to 0·2 μg/ml was obtained in the c.s.f. samples collected by the end of 2 h infusion, but when infusion was continued the curare-like activity in the c.s.f. samples collected by the end of the third and

TABLE 3. *Concentration of tubocurarine in consecutive hourly samples of c.s.f. obtained from dogs during a continuous intravenous infusion of* 10 (μg/kg)/min *tubocurarine (Tc)*

Experiment No.	Tc (μg/ml) 1 h	2 h	3 h	4 h
8	Nil	0·25	0·25	
9	0·05	0·35	0·30	0·25
12	0·38	0·40	0·60	0·75
13	0·12	0·40	0·20	
Mean	0·14	0·35	0·34	0·50

fourth hour decreased. The results obtained in four experiments on i.v. infusion at a rate of 10 (μg/kg)/min are summarized in Table 3, and the depression of the acetylcholine contractions of the frog rectus muscle obtained with 0·5 and 0·25 ml of the c.s.f. sample collected at the end of the 2 h infusion of experiment No. 13 is illustrated in Figure 3. Sample of c.s.f. collected before the intravenous infusion of tubocurarine did not depress the acetylcholine contractions. The concentration of tubocurarine in plasma samples taken at the same time as the c.s.f. samples are shown in Table 2. The values were higher than in the c.s.f. samples except in experiment No. 12, but since the values obtained for plasma were depressed by the potentiating effect of plasma on the acetylcholine contractions (see **Methods**) the actual tubocurarine concentration in the plasma samples of experiment No. 12 was probably also higher than in the c.s.f. samples.

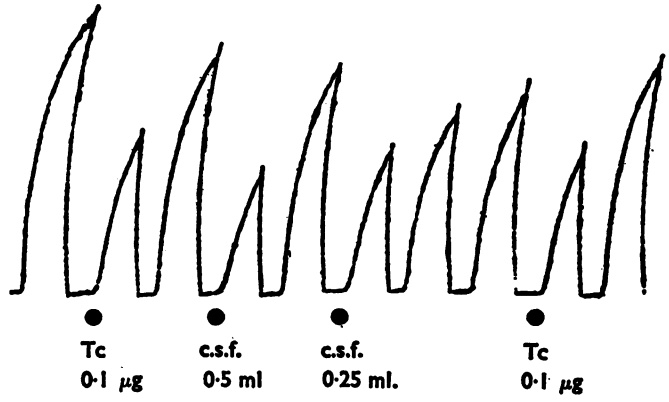

FIG. 3. Contractions of frog rectus muscle suspended in 1 ml bath to 0·15 μg acetylcholine. The black dots indicate that 2 min before the addition of acetylcholine the following additions were made to the bath: at the first and fourth dots tubocurarine (Tc) 0·1 μg, at the second and third dots 0·5 and 0·25 ml of c.s.f. collected from a dog at the end of 2 h intravenous infusion of tubocurarine at 10 (μg/kg)/minute. Same experiment as experiment 13 of Table 3.

Curare-like activity in cisternal effluent on perfusion from the lateral ventricle or from the lumbar subarachnoid space

When the cerebral ventricles were perfused from lateral ventricle to cisterna magna during an intravenous infusion of tubocurarine at a rate of 10 (μg/kg)/min the cisternal effluent collected during the first 15 min of infusion exhibited curare-like activity in four out of eight experiments, during the second 15 min period in 6, and during the next half hour in 7 out of 8 experiments. Thereafter curare-like activity was obtained in all samples of cisternal effluent. Expressed in terms of ng/min tubocurarine, the values varied greatly in the eight experiments which are summarized in Table 4, and in half of them they increased after the first hour of intravenous infusion. Figure 4 illustrates the effects of samples of cisternal effluent obtained from experiment No. 15 of Table 4, on the acetylcholine contractions of the frog rectus muscle. With 1 ml effluent collected before the intravenous tubocurarine infusion the acetylcholine contraction was accentuated. This effect was

TABLE 4. *Output of tubocurarine (Tc) in ng/min in cisternal or aqueductal effluent on perfusion from lateral ventricles obtained from dogs during intravenous infusion of* 10 *(μg/kg)/min tubocurarine*

Experiment No.	Tc output (ng/min) during consecutive 15, 30 and 60 min periods						
	15	15	30	60	60	60	60
2	16·7	10·7	17·3	25·0	33·3		
16*	Nil	Nil	6·7	6·5	11·3		
4	16·0	10·0	30·3	29·2			
10	Nil	21·3	17·3	20·0	16·0		
14	Nil	Nil	Nil	0·83	4·3	13·0	5·3
15	41·0	28·0	39·3	19·0			
3	10·0	10·0	19·0	14·3		5·7†	10·0†
5	Nil	13·3	20·0	21·7		25·0†	
6	Nil†	Nil†	3·0†	7·1†	9·1†		
7	Nil†	Nil†	16·0†	24·3†	13·0†		

* Weight of dog 8·2 kg. † Aqueductal effluent.

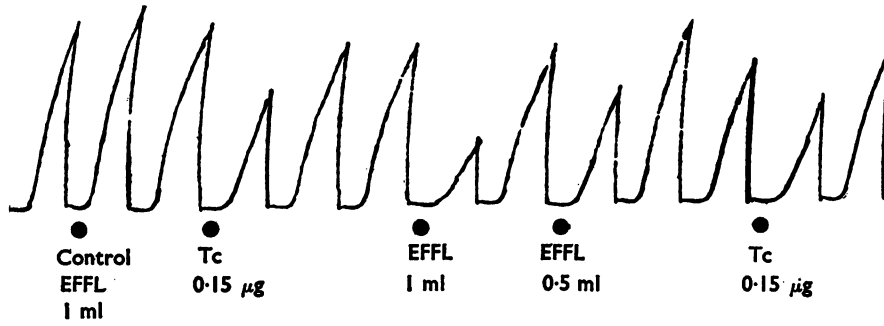

FIG. 4. Contractions of the frog rectus muscle suspended in 1 ml bath to 0·1 μg acetylcholine. The black dots indicate that 2 min before the addition of acetylcholine the following additions were made to the bath: at the second and fifth dots 0·15 μg tubocurarine (Tc), at the first dot 1 ml cisternal effluent (EFFL) collected from a dog before, and at the third and fourth dots 1 and 0·5 ml collected during the first 15 min of intravenous infusion of tubocurarine at 10 (μg/kg)/minute. Effluent collected during perfusion from lateral ventricle to cisterna. Same experiment as experiment 15 in Table 4.

obtained also in other experiments with control samples of cisternal or aqueductal effluent, but not in all experiments. On the other hand, both with 1 ml and 0·5 ml of the sample collected during the first 15 min of intravenous tubocurarine infusion, the acetylcholine contraction was depressed.

Table 5 summarizes results of five experiments in which the spinal subarachnoid space was perfused from the lumbar subarachnoid space to cisterna magna during an intravenous infusion of tubocurarine at a rate of 10 (μg/kg)/minute. In all

TABLE 5. *Output of tubocurarine (Tc) in ng/min in consecutive half-hour samples of cisternal effluent, on perfusion from lumbar subarachnoid space (A), and on perfusion from lateral ventricle (B), as well as of aqueductal effluent on perfusion from lateral ventricle (C) obtained from dogs during intravenous infusion of* 10 *(μg/kg)/min tubocurarine*

Experiment No.	Weight of dog in kg	Tc output (ng/min) in successive half-hour samples							
		A			B			C	
17	11·4	23·3	40·7	23·3	20·7	14·3			
18	13·0	Nil	21·3	13·3	26·7				
19	13·5	Nil	55·0	42·7	30·0	31·0			
20	10·0	18·7	16·0	18·8	14·3	11·3	—	6·7	9·0
11	11·7	Nil	6·7	6·3	15·7	35·0	—	15·0	

five experiments three successive half-hour samples of cisternal effluent were collected after the infusion was begun. Except in three of the first half-hour samples, curare-like activity was detected in all samples. When the activity is expressed in ng/min tubocurarine and compared with the values shown in Table 4 for cisternal effluent on perfusion from the lateral ventricle the activity is seen to be about the same, or a little greater.

In all five experiments of Table 5, the perfusion from the lumbar subarachnoid space was followed by one from the lateral ventricle to cisterna magna whilst the intravenous tubocurarine infusion was continued. The tubocurarine equivalents in the half-hour samples of cisternal effluent collected during this perfusion were about the same, some a little lower, some a little higher than those obtained during the preceding perfusion.

Curare-like activity in aqueductal effluent on perfusion from the lateral ventricle

The results of two experiments in which from the beginning the perfusion from lateral ventricle to aqueduct are included in Table 4, Nos. 6 and 7. No curare-like activity was detected during the first half-hour of intravenous infusion of tubocurarine at a rate of 10 (μg/kg)/min but the subsequent samples collected whilst the tubocurarine infusion continued showed curare-like activity. Expressed in terms of ng/min tubocurarine the activity was of the same order as in the cisternal effluent on perfusion from lateral ventricle. This is evident also from the experiments Nos. 3 and 5 of Table 4, in which perfusion was first from lateral ventricle to cisterna and then from lateral ventricle to aqueduct, as well as from the experiments Nos. 20 and 11 of Table 5, in which perfusion was first from lumbar subarachnoid space to cisterna, then from lateral ventricle to cisterna, and finally from lateral ventricle to aqueduct. In three of these four experiments the values for the aqueductal effluent were lower and in one the value was higher, than the values which were obtained for the last samples of cisternal effluent collected before the outflow was switched over to the aqueduct.

Intravenous injection of tubocurarine

Effects on respiration and arterial blood pressure

An intravenous injection of 0·3 mg/kg tubocurarine produced respiratory failure in 3–5 min and artificial ventilation had to be applied for 30 to 45 min before normal respiration returned. On intravenous injection of 3 mg/kg tubocurarine respiration failed immediately and artificial ventilation had to be continued throughout the experiment.

The intravenous injection of 0·3 mg/kg tubocurarine produced a transient fall in arterial blood pressure of about 30 to 40 mmHg. With 0·5 mg/kg blood pressure fell 60 to 80 mm, with 3 mg/kg it fell about 100 mmHg and recovery took over an hour. Pre-treatment with a few intravenous injections of 20 mg mepyramine given at 10 min intervals provided little protection against the hypotensive action of tubocurarine, and when such mepyramine injections were given during the prolonged fall produced by 3 mg/kg tubocurarine they brought about a transient rise of about 30 mmHg only.

TABLE 6. *Output of tubocurarine (Tc) in ng/min in cisternal effluent obtained from dogs on perfusion from lateral ventricle at different times after an intravenous injection of* 0·3 *or* 3 *mg/kg tubocurarine*

Experiment No.	Weight of dog in kg	Dose of tubocurarine (mg/kg)	Tc output (ng/min) 0–15 min	16–30 min	31–60 min	61–120 min
21	10·5	0·3	22 (0·66)	<20 (0·4)	Nil (0·25)	Nil (0·2)
22	9·1	0·3	24·6 (0·3)	6·6 (0·3)	Nil (0·17)	Nil (0·17)
23	9·5	3·0	40 (>1·0)	44 (>1·0)	60 (1·0)	44 (1·0)

The figures in parentheses refer to plasma concentration of tubocurarine in μg/ml.

Curare-like activity in cisternal effluent on perfusion from the lateral ventricle

Table 6 gives the results of three experiments in which a single intravenous injection of tubocurarine was made during perfusion of the cerebral ventricles from the lateral ventricle to the cisterna magna. In two experiments the dose injected was 0·3 mg/kg and in the third it was 3 mg/kg. The dose of 0·3 mg/kg is smaller and that of 3 mg/kg larger than the dose used in clinical studies as patients were given an intravenous injection of 30 mg which corresponded to between 0·52 and 0·66 mg/kg.

On injection of 0·3 mg/kg tubocurarine, curare-like activity was detected in the first 15 min sample of cisternal effluent collected after the injection; the activity of this sample corresponded to over 20 ng/min tubocurarine in both experiments. In the second 15 min sample the activity was greatly reduced and in the subsequent samples curare-like activity was no longer detected. On injection of 3 mg/kg, curare-like activity was detected in all samples of cisternal effluent collected up to 2 h after the injection. The activity corresponded to between 40 and 60 ng/min tubocurarine and was greatest in the sample collected during the second half-hour after the injection. In all three experiments the plasma concentration of tubocurarine, given in parentheses, was greatest during the first 30 min after the injection and then declined.

Discussion

The finding that samples of c.s.f. obtained from patients by lumbar puncture after an intravenous injection of tubocurarine exerted curare-like activity when they were tested on the acetylcholine contraction of the frog rectus muscle agrees with the results obtained in a patient by Mahfouz (1949) and in dogs by Dal Santo (1964). These authors also found curare-like activity in c.s.f. collected after an intravenous injection of curare, but the values obtained by Mahfouz were nearly ten times greater than those found in the present experiments although the intravenous dose of tubocurarine he injected was 2·5 to 3 times smaller. Cohen (1963) who used a chemical method for estimating curare, failed to detect any in c.s.f. collected from patients after they were given an intravenous injection of tubocurarine. If the curare-like activity produced by human c.s.f. on the frog rectus muscle was not due to tubocurarine itself but to a derivative which is formed during its passage from blood into c.s.f., but which retains its biological activity, the difference in the results would be explained. This kind of explanation would not apply, however, to the negative results obtained by Bhattacharya & Feldberg

(1958b) when they studied the passage of anticholinesterases from blood into c.s.f. in anaesthetized cats. In these studies in which the cerebral ventricles were perfused from lateral ventricle to cisterna, 1 to 10 mg/kg was sometimes injected intravenously. Yet no curare-like activity was exerted on the frog rectus muscle with the effluent collected after the injection. This negative result is in contrast to the positive results obtained in the present experiments on anaesthetized dogs. This difference could be due to a difference in species. Another explanation could be that in the experiments of Bhattacharya & Feldberg, a potentiating effect exerted on the acetylcholine contraction of the frog rectus muscle by anticholinesterase present in their samples of cisternal c.s.f. had masked a depressant effect of tubocurarine also present in the samples.

Curare is a quaternary ammonium compound and is expected to cross the blood-c.s.f. barrier with difficulty. The passage of hexamethonium, another quaternary ammonium compound, from blood into c.s.f. has been tested by Paton (1952) in anaesthetized cats. After repeated intravenous injections of hexamethonium to maintain a plasma concentration of 50 to 100 μg/ml the c.s.f. contained less than 1 μg/ml. In the present experiments on anaesthetized dogs the tubocurarine concentration in the c.s.f. was of the same order after an intravenous infusion of 10 (μg/kg)/min but the plasma concentration of tubocurarine attained was much lower than that of hexamethonium. With the exception of one experiment it was never higher than 1 μg/ml. This would suggest that tubocurarine passes more readily than hexamethonium into the c.s.f. This may again be due to a difference in species or it may be that curare causes a partial breakdown of the barrier. As curare releases histamine, this could be an action of the released histamine. However, the amounts released in the experiments on the anaesthetized dogs cannot have been great. The hypotension produced by tubocurarine was probably not due to released histamine because it was not abolished by mepyramine. It was probably due to ganglionic blockade and the resultant cardiovascular effects as suggested by Smith, Proctor & Spence (1970) and by Hughes (1970). Furthermore, gallamine, which does not cause histamine release or hypotension behaved like tubocurarine. On its intravenous injection into dogs it passed into the cisternal c.s.f. (Haranath, Krishnamurty & Seshagirirao, unpublished experiments).

In previous experiments in which adrenaline was infused intravenously into anaesthetized cats (Draskoci, Feldberg & Haranath, 1960) or atropine into anaesthetized dogs (Haranath, *et al.*, 1966) whilst the liquor space was perfused from lateral ventricle to either aqueduct or cisterna magna the concentration of these substances was greater in the cisternal than in the aqueductal effluent. The results of the present experiments are not so clear-cut. In the two experiments in which, from the beginning, perfusion was from lateral ventricle to aqueduct the curare-like activity in the aqueductal effluent was within the range, though perhaps at the lower end of the values obtained for cisternal effluent when perfusion was from lateral ventricle to cisterna. On the other hand, in three out of four experiments in which a comparison of cisternal and aqueductal effluent could be made in the same experiment because perfusion from lateral ventricle was first to cisterna and then to aqueduct, the curare-like activity was greater in the cisternal than in the aqueductal effluent. Further, some of the highest values for curare-like activity were obtained in cisternal effluent on perfusion from the lumbar subarachnoid

space. These results as well as the finding of curare-like activity in lumbar c.s.f. obtained from patients after intravenous injection of tubocurarine practically exclude secretion of tubocurarine into the liquor space from the choroid plexus as the origin of the curare-like activity in the c.s.f. or in the effluent on perfusion of the different regions of the liquor space.

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