Br. J. Pharmac. Chemother. (1966), **27**, 10–16.

PASSAGE OF INTRAVENOUSLY INFUSED ATROPINE INTO PERFUSED CEREBRAL VENTRICLES AND SUBARACHNOID SPACE

BY

P. S. R. K. HARANATH, K. PREMALATHA AND K. SUNANDA-BAI

From the Department of Pharmacology, Kurnool Medical College, Kurnool A.P., India

(*Received September* 17, 1965)

Stern & Gautier (1921) detected atropine in the cerebrospinal fluid of the dog and of the rabbit, after its intravenous or intracarotid injection. The present experiments were undertaken to see if in the cat also atropine passes from the blood stream into the cerebrospinal fluid, and if so, to determine the rate of its output in the effluents obtained on perfusion of various parts of the cerebral ventricles and subarachnoid space.

Our experiments with cats show that atropine, during its intravenous infusion, appears in the cerebrospinal fluid, and further it was found in the effluents from both the perfused cerebral ventricles and the perfused subarachnoid space. There was no significant difference between the output from the cerebral ventricles and that from the subarachnoid space.

METHODS

Cats weighing 1.5 to 4 kg were anaesthetized with chloralose, 70 mg/kg, injected intravenously under ethyl chloride and ether anaesthesia. The trachea was cannulated. For the atropine infusion a cannula was inserted in the right femoral vein. The atropine sulphate in 0.9% saline was infused at 0.2 ml./min with a continuous slow injector at rates of 1, 10 or 25 μg/kg/min. Blood samples were obtained from the left femoral artery, which was dissected free and clamped; an opening was made in the artery for the insertion at intervals of 1 hr of a polythene tube, through which the samples were collected into tubes containing heparin, and then immediately centrifuged and the plasma separated. The plasma was kept at room temperature (25 to 30° C) if assayed on the same day, or in the refrigerator (at 4° C) for assay on the next day.

Collection of cerebrospinal fluid. A cisternal cannula similar to one described by Bhawe (1958) was inserted into the cisterna magna. The cannula consisted of a 20 S.W.G. hypodermic needle with the butt removed and a small length of polythene tube attached. The free end of the polythene tube was closed with a small glass stillete. Cerebrospinal fluid samples were collected every hr by removing the glass stillete and allowing 0.5 to 1 ml. of cerebrospinal fluid to flow into a test tube. The stillete was then replaced.

Perfusion of the cerebral ventricles and subarachnoid space. A Collison cannula was implanted into the lateral ventricle as described by Feldberg & Sherwood (1953). Perfusion from the lateral ventricle to the cisterna magna or to the aqueduct was then carried out as described by Bhattacharya & Feldberg (1958).

The spinal subarachnoid space was perfused from the lumbar region to the cisterna magna. To introduce the inflow cannula, a polythene tube of 1 mm bore, the arches of the last two lumbar

vertebrae were removed and an opening made in the exposed meninges. The cannula was then pushed through this opening into the spinal subarachnoid space for a distance of 20 to 40 mm and tied in position. The outflow was collected from a cannula placed in the cisterna magna as described above.

To compare the output of atropine from the cerebral ventricles and the subarachnoid space in the same experiment, the following procedures were adopted. In some experiments the spinal subarachnoid space was first perfused for 2 hr and then the inflow was changed from the lumbar subarachnoid cannula to a cannula previously placed in the lateral ventricle. The outflow was collected still from the cisterna magna, until one hr later, when the cisternal drainage cannula was replaced by an aqueductal drainage cannula for the last hr. In other experiments perfusion was from the lateral ventricle throughout, and outflow was taken from the cisterna magna for the first 1 to 3 hr, and then from the aqueduct.

The fluid used for perfusion was that described by Leusen (1949). The rate of perfusion was 0.1 ml./min throughout. The effluent was usually collected in 20 min samples.

Assay of atropine. Perfusate, plasma and cerebrospinal fluid were assayed for atropine on the acetylcholine contractions of guinea-pig ileum as described by Burn (1952). The acetylcholine contractions were usually significantly depressed by 0.02 μg atropine sulphate added to the bath 1 min before the acetylcholine ; sometimes the preparation was sensitive to 0.01 μg atropine. The samples of perfusate and plasma were assayed on the day of collection or next day. In preliminary experiments the results obtained when an assay was repeated on the next day were the same as on fresh samples. The results are given in terms of atropine sulphate.

Plasma, to which atropine was added *in vitro* to a concentration of 0.2 μg/ml., was assayed 1 hr later, and the concentrations of atropine found ranged from 0.08 to 0.2 μg/ml. and did not fall thereafter.

Undiluted plasma had sometimes to be added to the bath in volumes up to 1 ml. When added to the bath in volumes of more than 0.2 ml., undiluted plasma produced a small contraction by itself, and also potentiated the acetylcholine contractions of guinea-pig ileum, thus masking the depressant action of the atropine present in it. But the acetylcholine contractions elicited 2 min later were still depressed if atropine was present in the sample.

RESULTS

Plasma atropine concentrations

Samples of plasma taken hourly during the intravenous infusion of atropine at rates of 1, 10 or 25 μg/kg/min showed atropine-like activity ; the results are given in Table 1. In only two of the five experiments with infusion at 1 μg/kg/min was definite atropine-like activity found. In the other three experiments either a trace or no atropine-like activity was detected. As seen in Table 1, with 10 μg/kg/min atropine, the plasma levels ranged widely, from 0.04 to 1.2 μg per ml., and exceeded in several experiments levels obtained with 25 μg/kg/min.

Appearance of atropine in the cerebrospinal fluid

Samples of cerebrospinal fluid were collected from the cisterna magna in one experiment before and during the intravenous infusion of atropine 10 μg/kg/min. The cerebrospinal fluid collected during, but not before, the infusion of atropine showed atropine-like activity. The concentration of atropine found in the cerebrospinal fluid, which rose steadily to reach 0.06 μg/ml., and the corresponding blood levels are shown in Table 2.

TABLE 1

PLASMA CONCENTRATIONS OF ATROPINE DURING ITS INTRAVENOUS INFUSION

Rate of atropine infusion μg/kg/min	Exp. no.	Weight of cat (kg)	Atropine (μg/ml.) in successive hourly samples 1	2	3	4	mean
1	5	2·1	0·037	0·033	0·02		0·03
	1	2·8	0·08	0·2	0·05	<0·05	0·11
10	19	2·5	0·05	0·04	0·06		0·05
	9	3·4	0·06				0·06
	8	2·7	—	0·06	0·05	0·08	0·063
	16	2·5	0·08	0·133	0·05	0·1	0·091
	18	2·9	0·08	0·08			0·08
	20	2·4	0·066	0·1	0·2	0·1	0·117
	13	2·6	0·4	0·15	0·15		0·233
	7	2·5	0·3	0·3	0·5	0·3	0·35
	17	2·0	1·2	0·53	0·23	0·23	0·548
25	21	2·4	0·30	0·4	0·05	0·3	0·262

TABLE 2

ATROPINE CONCENTRATIONS IN C.S.F. AND PLASMA DURING INTRAVENOUS INFUSION OF 10 μG/KG/MIN

	Atropine concentration, μg/ml., in consecutive hourly samples 1	2	3	4
C.S.F.	0·025	0·03	0·04	0·06
Plasma	1·2	0·53	0·23	0·23

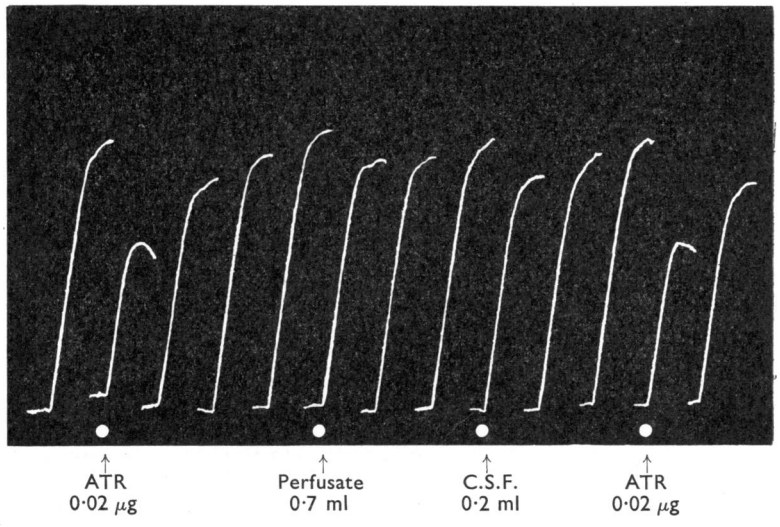

Fig. 1. Effects of control perfusate, c.s.f. and atropine on ACh contractions of guinea-pig ileum suspended in a 10 ml. bath. All contractions are due to 0.5 μg ACh, which was added every 2 min and left in the bath for 30 sec. At the white dots: the contractions 1 min after the addition of the test solutions.

Appearance of atropine in the effluent from the perfused subarachnoid and ventricular spaces

The effluents collected by the various techniques used before starting the atropine infusions had generally either a potentiating action or no effect on the acetylcholine contractions of the guinea-pig ileum. In a few instances slight depression of acetylcholine contractions was observed, which was considerably less than the depression produced by 0.01 to 0.05 μg atropine. Fig. 1 shows the depressant action of a control perfusate and of cerebrospinal fluid from one such experiment compared with that of atropine.

Perfusion from lateral ventricle to cisterna. Atropine was present in the cisternal effluent from the first sample during intravenous infusion of 1, 10 or 25 μg/kg/min.

TABLE 3

ATROPINE OUTPUT IN CISTERNAL OR AQUEDUCTAL (bold figures) EFFLUENT FROM THE PERFUSED CEREBRAL VENTRICLES DURING ITS INTRAVENOUS INFUSION

Rate of atropine infusion μg/kg/min	Exp. no.	Weight of cat (kg)	Atropine output (ng/min) in successive half hourly samples										mean cistern. output
			1st hr		2nd hr		3rd hr		4th hr		5th hr		
1	1	2·8	2·2	2·3	2·9	4·0	1·7	2·3	4·1	4·1			3·0
	2	2·0	6·3	6·9	5·6	3·7	3·8	2·3	1·9	1·9			4·1
	3	2·5	1·4	2·4	2·9	2·0	1·8	2·1					2·1
	4	2·7	4·3	4·3	2·4	2·6		**1·9**	**2·0**				3·4
10	6	4·0	2·7	2·6	2·4	4·4	2·5	2·5					2·9
	7	2·5	14·1	21·6	11·3	10·1	10·2	17·9	23·5	19·0			16·0
	8	2·7	6·4	4·5	3·9	5·0	5·4	5·8	3·7	4·0			4·8
	9	3·4	3·8	7·8									5·8
	10	2·5					3·3	4·3	6·0				4·5
	11	2·7	4·2	3·8	4·9	6·3							4·8
	12	3·0	2·3	3·9	2·5	1·9	2·0		*Nil*	*Trace*	*Trace*		2·5
	13	2·6	3·9	4·6	3·9	3·8					**<1·8**	**<1·5**	4·1
25	21	2·4	2·8	4·9	5·5	12·9	6·7	6·3	5·5	9·1	**6·6**		6·7

The rate of output in the first hr was generally maintained during the rest of the experiment with some fluctuation.

When 1 μg/kg/min was infused, the output of atropine in the cisternal effluent was low, as shown in each of the experiments summarized in Table 3. The output was usually between 2 and 5 ng/min. The maximum found was less than 7 ng, and in three experiments either a trace of or no atropine-like activity could be detected in the plasma.

With infusion of 10 μg/kg/min the output of atropine ranged from a trace to nearly 25 ng/min, but usually lay between 2 and 10 ng/min. These results are shown in Table 3.

Infusion of atropine at the rate of 25 μg/kg/min in one experiment did not yield a substantially greater output in the cisternal effluent than the infusions of 10 μg/kg/min. This result, given in the last line of Table 3, might be attributed to the associated low plasma level shown in Table 1.

Perfusion of the spinal subarachnoid space. The spinal subarachnoid space was perfused for 2 hr in three experiments during infusion with 10 μg/kg/min atropine, before perfusing from the lateral ventricle. The output of atropine found in the effluent ranged from 2.1 to 7.9 ng/min. These results are given in Table 4.

TABLE 4

ATROPINE OUTPUT WITH SUCCESSIVE PERFUSION (a) OF THE SPINAL SUBARACHNOID SPACE, (b) FROM LATERAL VENTRICLE TO CISTERNA MAGNA, and (c) FROM LATERAL VENTRICLE TO AQUEDUCT (in bold figures), DURING INTRAVENOUS INFUSION OF ATROPINE 10 μG/KG/MIN.

		Atropine output (ng/min) in successive half hourly samples during perfusions								
		(a) Of Spinal subarachnoid space				(b) From lat. ventricle to cisterna		(c) From lat. ventricle to aqueduct		
Exp. no.	Weight of cat (kg)	1st hr		2nd hr		3rd hr		4th hr		5th hr
14	2·8	2·5	4·4	4·5	3·7	4·2				
15	1·5	2·3	3·9	7·9	4·6	2·4	3·3	—	**3·5**	**3·8**
16	2·5	2·4	2·6	4·4	3·2	3·5	2·1	—	**<1·9**	**<2·3**

In each of these experiments the inflow was changed over to a cannula previously placed in the lateral ventricle after the 2 hr perfusion of the spinal subarachnoid space. As shown in Table 4 the output of atropine did not then differ appreciably from the preceding output collected from the spinal subarachnoid perfusion.

Perfusion from the lateral ventricle to aqueduct. The outputs of atropine from the aqueduct were low, and ranged between a trace and 6.6 ng/min. These results are given in bold figures in Tables 3 and 4. The maximum output was obtained in experiment 21 (Table 3), with atropine infused at 25 μg/kg/min. It can be seen that, in the same experiment on perfusion from the lateral ventricle, the output from the aqueduct was generally lower than that in the cisternal effluent. The output from the aqueduct was also (in experiments 15 and 16 of Table 4) slightly less than from the spinal subarachnoid space.

DISCUSSION

The present experiments show that atropine passes from the blood stream into the cerebrospinal fluid in cats. That this passage can take place even at low plasma atropine concentrations is borne out by the presence of atropine in the effluent when only 1 μg/kg/min was infused. On the other hand high plasma levels were not always associated with proportionately greater output into the effluent, suggesting that this passage is not a simple process.

Stern & Gautier (1921), in their experiments to determine if atropine passed from the blood to the cerebrospinal fluid, employed very large doses. They found atropine-like activity in the cerebrospinal fluid after administering 720 mg atropine in 7 min into the femoral vein in the dog, and 60 mg into the carotid artery in the rabbit. But after they injected 60 mg atropine into the ear vein of a rabbit, no atropine was found in the cerebrospinal fluid. In our experiments small amounts of atropine were infused into a vein ; the total amounts of atropine infused over a period of 4 hr in our experiments were about 700 μg with 1 μg/kg infusion/min, and 10 mg with 10 μg/kg/min. These amounts may also seem large when compared to human therapeutic doses, but were much smaller than the doses used by Stern & Gautier (1921) and were well tolerated by the cats.

The recovery of atropine from the cerebral ventricles was only slightly less than from the subarachnoid space. It is therefore concluded that there is no significant difference in the output of atropine from the cerebral ventricles and from the subarachnoid space. This recalls the finding of Draskoci (1960), that during the intravenous infusion of 10 μg/kg/min lysergic acid diethylamide there was no obvious difference in the output of the diethylamide from the perfused cerebral ventricles, whether the perfusion was from lateral ventricle to cisterna or from lateral ventricle to aqueduct, that is, included or excluded part of the subarachnoid space. Draskoci, Feldberg & Haranath (1960) showed, in experiments similar to ours, that during intravenous infusion of adrenaline 40 μg/kg/min, more of the amine appeared in the effluent from the perfused subarachnoid space than from the perfused cerebral ventricles.

Atropine could enter the cerebral ventricles either from the choroid plexuses or through the brain substance; it could reach the cranial subarachnoid space directly either through the substance of the brain, or the tufts of choroid plexus projecting out of the fourth ventricle, or from the meningeal vessels ; it could enter the spinal subarachnoid space directly only through the substance of the spinal cord or the meningeal vessels. Since there are no significant differences in the outputs of atropine into these different compartments, it is likely that its passage from the bloodstream into the cerebrospinal fluid is mainly through the neural substance common to all these spaces.

SUMMARY

1. In cats under chloralose anaesthesia samples of blood and c.s.f. were taken, and the cerebral ventricles and the subarachnoid space were perfused during continuous intravenous infusion of atropine 1, 10 or 25 μg/kg/min.

2. The plasma concentrations of atropine during intravenous infusion were variable. With 1 μg/kg/min plasma concentrations were 0 to 0.2 μg/ml. ; with 10 μg/kg/min, 0.05 to 1.2 μg/ml., and with 25 μg/kg/min 0.05 to 0.3 μg/ml.

3. Samples of c.s.f. collected hourly in one experiment during intravenous infusion of 10 μg/kg/min atropine showed progressively increasing concentrations of atropine from 0.025 μg to 0.06 μg/ml.

4. On perfusion from lateral ventricle to cisterna magna, the output of atropine during infusion of 1 μg/kg/min was up to 7 ng/min, with infusions of 10 μg/kg/min 2 to 24 ng/min, and with infusions of 25 μg/kg/min 2.8 to 12.9 ng/min.

5. On perfusion from lateral ventricle to aqueduct the atropine output ranged from a trace to 6.6 ng/min.

6. On perfusion of the spinal subarachnoid space, during intravenous infusion of 10 μg/kg/min atropine, the output was 2.3 to 7 ng/min.

7. There were no significant differences in the output whether the perfusions were from lateral ventricle to cisterna or aqueduct, or when the spinal subarachnoid space was perfused.

This investigation was carried out with the aid of a State Medical Research Grant from Government of Andhra Pradesh, India.

REFERENCES

BHATTACHARYA, B. K. & FELDBERG, W. (1958). Perfusion of the cerebral ventricles: effects of drugs on outflow from cisterna and aqueduct. *Br. J. Pharmac. Chemother.*, **13**, 156–162.

BHAWE, W. B. (1958). Experiments on the fate of histamine and acetylcholine after their injection into cerebral ventricles. *J. Physiol. (Lond.)*, **140**, 169–189.

BURN, J. H. (1952). *Practical Pharmacology*. Oxford: Blackwell Scientific.

DRASKOCI, M. (1960). Assay of lysergic acid diethylamide and its passage from blood into the perfused cerebral ventricles. *Br. J. Pharmac. Chemother.*, **15**, 29–34.

DRASKOCI, M., FELDBERG, W. & HARANATH, P. S. R. K. (1960). Passage of circulating adrenaline into perfused ventricles and subarachnoid space. *J. Physiol. (Lond.)*, **150**, 34–49.

FELDBERG, W. & SHERWOOD, S. L. (1953). A permanent cannula for intraventricular injections in cats. *J. Physiol. (Lond.)*, **120**, 3P.

LEUSEN, I. (1949). The influence of calcium, potassium and magnesium ions in cerebrospinal fluid on vasomotor system. *J. Physiol. (Lond.)*, **110**, 319–329.

STERN, L. & GAUTIER, R. (1921). Rapports entre le liquide céphalorachidien et la circulation sanguine. *Arch. int. Physiol.*, **17**, 138–192.