RESEARCH PAPERS

ORAL AND BRONCHIAL FLUIDS IN POISONING WITH ANTICHOLINESTERASES

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PROFUSE secretion of fluid from mouth, nose and eyes is a frequently observed sign in acute poisoning with anticholinesterases, and the accumulation in the airway of this fluid may aggravate the respiratory distress caused.

The present investigation reports preliminary studies on the origin of this secretion. In order to obtain quantitative information, fluids flowing from the cannulated trachea and also from the mouth (and nose) of anæsthetised rabbits, cats and monkeys were collected before and after the administration of anticholinesterase or parasympathomimetic agents.

Only small increases of the normally very low flow of bronchial secretion were found after *iso*propylmethylphosphorofluoridate (Sarin), *N-p*-chlorophenyl-*N*-methyl carbamate of *m*-hydroxy phenyltrimethylammonium bromide (Nu 1250), and di*iso*propylphosphorofluoridate (dyflos); pilocarpine increased the bronchial fluid flow considerably; 5-(2 methyl-1 : 3-dioxa*cyclo*pentane) choline (2268 F) had no effect at all. Salivary secretion was significantly increased after large doses of the anticholinesterases and abundant after therapeutic doses of parasympathomimetics.

METHODS

Nineteen rabbits, 7 cats, and 2 monkeys were used. The anæsthetics were varied; pentobarbitone, urethane or chloralose were employed. The animals were held in the prone position in a V-shaped elevated trough inclined towards the head end at 15 to 20° to the horizontal. The legs could be fastened to the vertical stands of the trough.

Collection of Bronchial Fluid

To facilitate the free flow of bronchial fluid the animal must breathe warm moist air but rebreathing with accumulation of CO_2 has to be avoided. A free modification of the apparatus employed by Perry and Boyd[1] was used to collect the bronchial fluid (Fig. 1). One limb of a short 4-way cruciform cannula was inserted in the trachea just below the larynx. The animal was then transferred to the trough and a current of air at 41° C. and saturated with water vapour at this temperature was passed through the now horizontal limbs of the cannula. The animal could breathe freely from this current of air while all expired air was swept away in the current so that rebreathing artefacts were avoided. The lower part of the vertical limb, carrying droplets of bronchial fluid

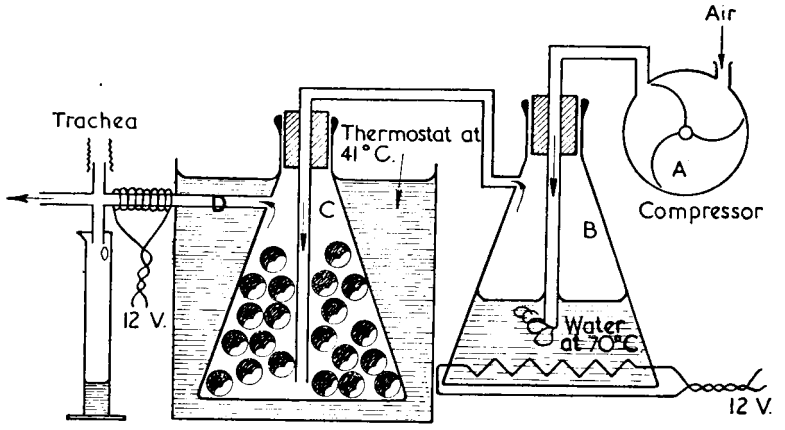

FIG. 1. Apparatus for the collection of bronchial fluid.

and some spurious condensate, was fitted into a small measuring cylinder. A diagram of the arrangement is shown in Fig. 1. The current of air (4 to 5 l./min.) was provided by the small compressor (A) and was bubbled through water in flask (B). The water in this flask was maintained at 60° C. by means of a low-voltage immersion heater. The outgoing air, partially saturated with water vapour at 60° C. was led into vessel (C). This vessel was immersed in a water bath which was thermostatically controlled and kept at a temperature of 41° C. The vessel was filled with pieces of broken glass and here the excess water vapour could condense and the air reach a temperature of 41° C. while it was fully saturated with water at this temperature. The tube (D) leading from this vessel to the trachea of the animal was lagged with an electrically heated flex and kept at 42 to 45° C. In this way all condensation in the tube was avoided.

Collection of Fluid from Mouth and Nose

Copious secretions from these sites occur frequently and collection was simple. The hanging head of the animal was resting in a glass funnel and the stem of the funnel was placed in a measuring cylinder. In some cases it was necessary to keep the mouth open by an inserted wire ring.

Readings of the accumulated volume of fluid were taken at hourly or shorter intervals. From these data fluid flow rates were calculated as ml./hr./kg. To assess the effect of a treatment the maximum flow rate obtained during or after the treatment was related to the mean pre-treatment flow rate of the same animal.

RESULTS

Pre-Treatment Flow-rates

Before commencing any treatment, flowrates were observed for a period of two hours in order to obtain a "normal baseline" for each animal. Flowrates of oral and bronchial fluid are approximately 0·1 to 0·3 ml./hr./kg. and are similar for rabbit, cat and monkey. In the cat and the

rabbit the oral fluid flow is two to three times greater than the bronchial flow. Rabbits produce slightly more fluid than cats. Most of the variation is due to difference between animals; the flowrates of each individual animal do not change greatly over a period of one to four hours. Bronchial fluid in rabbits is thin, almost colourless and only slightly opalescent. In cats, globules of thick green mucus are not uncommon.

Sarin

Four cats were injected subcutaneously with 12 to 25 μg./kg. doses of sarin, repeated at half hourly intervals, until general symptoms appeared. In three out of the four cats slight convulsions, dyspnœa or arrest of respiration occurred after a total dose of 50 to 60 μg./kg. of sarin had been given. (In the fourth cat this happened after 25 μg./kg.) An increased oral fluid flow occurred only when signs of sarin poisoning were evident and the rate of flow was roughly proportional to the severity of the symptoms. Thus the onset of the increased flow was always accompanied by extensive skin twitching and reached its height during the muscular fasciculations and general convulsions. (To prolong the life of the animals, positive pressure respiration was given at the first signs of an arrest of respiration.) When these general symptoms had appeared the oral fluid flow rate was increased to a maximum of 3·7 ml./hr./kg., i.e., approximately 18 times the pre-treatment rate. The bronchial fluid flow was only slightly increased to a maximum of 0·08 ml./hr./kg., i.e., to not quite twice the pre-treatment rate.

Rabbits were found to yield smaller volumes of fluid than cats after sarin. Repeated subcutaneous doses of between 10–20 μg./kg. were given to four rabbits. After a mean dose of 34 μg./kg. general symptoms developed and the oral fluid rate increased to a maximum of 0·46 ml./hr./kg., i.e., to 2·4 times the pre-treatment value. The corresponding value for the bronchial fluid was 0·13 ml./hr./kg. (three times the pre-treatment mean). One monkey had a subcutaneous dose of 70 μg./kg. followed by 17 μg./kg. repeated four times at half hourly intervals. After a total dose of 138 μg./kg. general muscular twitching developed and simultaneously the oral fluid rate increased to a maximum of 9 ml./hr./kg. (300 times the pre-treatment mean). The bronchial fluid reached a maximum of 0·24 ml./hr./kg. (nearly twice the pre-treatment mean). A further dose of 17 μg./kg. of sarin at this stage prolonged the increased flow but did not augment it.

Sarin Mixed with Acetylcholine

In sarin poisoning the twitching and fasciculations of the muscles seemed to be the necessary preliminary for the commencement of an increased flow. It could be assumed that the acetylcholine formed in the course of this muscular activity builds up a gradually increasing blood level which reinforces the acetylcholine formed locally in the salivary gland[2].

To simulate a fast build-up of acetylcholine, mixtures of sarin and acetylcholine were administered in small doses repeated at regular intervals. Considerably increased naso-oral flow rates are obtained in

this way. The onset is very gradual at first but after 6 or 7 preliminary doses the flow suddenly increases to a relatively high rate. Six rabbits received 8 half-hourly doses of 7·5 μg./kg. sarin mixed with 60 μg./kg. acetylcholine. The maximum effect on oral flow rate occurred after a total of 50 μg./kg. of sarin had been given; this maximum was 7·5 ml./hr./kg. (29 times the pre-treatment rate). The bronchial flow rate was only slightly affected with a maximum of 0·18 ml./hr./kg. (1·5 times the pre-treatment rate). One cat, after 3 half-hourly doses of 10 μg./kg. sarin mixed with 100 μg./kg. acetylcholine produced a maximal oral flow of 21 ml./hr./kg. (269 times the pre-treatment rate). The maximal bronchial flow was 0·6 ml./hr./kg. (19 times the pre-treatment rate). One monkey treated with 2 half-hourly subcutaneous doses of a mixture of sarin (6 μg./kg.) and acetylcholine (60 μg./kg.) developed a maximal oral flow of 9·8 ml./hr./kg. (82 times the pre-treatment rate) and a bronchial flow maximum of 0·12 ml./hr./kg. or twice the pre-treatment rate. Three further doses of 6 μg./kg. of sarin + 60 μg./kg. of acetylcholine at half-hourly intervals prolonged the response without further augmenting it.

Nu 1250

This substance inhibits the "true" cholinesterase predominantly. It has been shown to produce regularly a copious spontaneous secretion of saliva after close-arterial injection into the submaxillary gland[2]. In one rabbit, after a single subcutaneous dose of 0·2 mg./kg. of Nu 1250 a maximal oral fluid flow of 42 ml./hr./kg. (127 times the pre-treatment rate) developed during the violent terminal convulsions lasting 5 minutes. Simultaneously the bronchial fluid flow increased to 1·2 ml./hr./kg. (10 times the pre-treatment rate). The second rabbit, after a dose of 0·5 mg./kg. of Nu 1250, did not convulse and maximal fluid flow rates were much lower, viz., 1·3 ml./hr./kg. for the oral, 0·12 ml./hr./kg. for the bronchial flow, i.e., 26 times and twice the pre-treatment rates respectively.

Dyflos and Dyflos Mixed with Acetylcholine

One rabbit received seven doses of 0·1 mg./kg. dyflos at half-hourly intervals. Oral and bronchial fluid flows remained approximately at pre-treatment rates. A second rabbit, after two doses of 0·5 mg./kg. dyflos showed an increase in oral fluid to 2·5 ml./hr./kg. (8·6 times the pre-treatment rate) while the bronchial flow decreased to half the pre-treatment rate.

A mixture of dyflos and acetylcholine was tried in one cat. After six doses of 0·1 mg./kg. dyflos + 50 μg. of acetylcholine followed by 4 doses of 0·2 mg./kg. dyflos + 0·1 mg./kg. of acetylcholine the oral fluid flow reached a maximum of 5·1 ml./hr./kg. (34 times the pre-treatment rate) whereas the bronchial fluid maximum was only 0·14 ml./hr./kg. (twice the pre-treatment rate).

Pilocarpine and 2268 *F*

These parasympathomimetic agents produce by far the most dramatic effects on oral fluid flow at doses which are not close to the LD50, but

whereas pilocarpine increases the bronchial flow substantially, 2268 F does not produce an increase. Pilocarpine in a single dose of 6·2 mg./kg. to a cat produced a sudden increase of oral flow to a maximum of 24 ml./hr./kg. with a simultaneous sharp rise of bronchial fluid flow to a maximum of 0·7 ml./hr./kg.

2268 F in a single dose of 200 μg./kg. to a rabbit gave, within a few minutes, a maximum oral flow of 43 ml./hr./kg. but had no effect at all on the bronchial fluid. Another rabbit had three subcutaneous doses of 20 μg. of 2268 F at half-hourly intervals. A maximum oral fluid flow of 49 ml./hr./kg. was reached and within the next 3 hours a total of 240 ml. of oral fluid was collected. Again there was no effect at all on the bronchial fluid flow.

Acetylcholine

Repeated doses of acetylcholine by itself were given subcutaneously to two rabbits as a control to the experiments with mixtures of sarin and acetylcholine. Seven half-hourly doses of 50 μg. of acetylcholine per kg. did not alter the pre-treatment rate of flow of either oral or bronchial fluids. Seven 500 μg. doses of acetylcholine given at half-hourly intervals produced erratic increases to a maximum of 0·7 ml./hr./kg. for the oral and 0·2 ml./hr./kg. for the bronchial flow.

Discussion

Two essential points are borne out by the results. Firstly, all the anticholinesterases and parasympathomimetics that were tried had a much greater effect on the oral, than on the bronchial flow. Secondly, in poisoning with anticholinesterases fluid flow rates increased in parallel with the severity of the general signs of the poisoning. If the dose was not large enough to cause at least skin twitching, flow rates remained at pre-treatment levels. Sarin administered subcutaneously to conscious

TABLE I

Summary of effect of various anticholinesterases on oral and bronchial fluid flow

Species and No. used	Agent	Mean total dose in μg.	Oral fluid	Bronchial fluid
			(The figures are ratios of flowrates before and after treatment)	
4 Rabbits	Sarin	34	2·8	3·0
4 Cats	Sarin	47	18·3	1·4
1 Monkey	Sarin	138	30·7	1·9
6 Rabbits	Sarin	51	29·0	1·5
	Acetylcholine	442		
1 Cat	Sarin	30	296·0	19·0
	Acetylcholine	300		
1 Monkey	Sarin	12	81·7	2·0
	Acetylcholine	120		
1 Rabbit	Acetylcholine	350	1·0	1·0
1 Rabbit	Acetylcholine	3500	2·3	1·8
2 Rabbits	Dyflos	850	4·5	1·0
1 Cat	Dyflos	1400	34·0	2·0
	Acetylcholine	700		
2 Rabbits	Nu 1250	350	76·8	5·0
2 Rabbits	2268 F	130	88·0	0·8
1 Rabbit	Pilocarpine	6·4	66·2	3·1
1 Cat	Pilocarpine	6·2	100·0	14·4

dogs provokes intense salivary secretion usually only at doses which cause general convulsions. A slightly increased flow of saliva appears shortly before the onset of the convulsions, but the animal can usually swallow this secretion. As the convulsions become more intense, copious saliva escapes from the animal's mouth (Spencer, oral communication).

It is interesting to note the difference on bronchial flow between 2268 F and pilocarpine. Both agents have a powerful action on salivary secretion, the smooth muscles of stomach, intestine and urinary bladder, and a similar miotic action[3]; yet only pilocarpine increases the bronchial fluid flow[4]; 2268 F leaves it unaffected.

A summary of the mean experimental results is shown in Table I.

Summary

1. An attempt has been made to obtain quantitative data on oral and bronchial fluid flow in poisoning with anticholinesterases and parasympathomimetics.

2. All anticholinesterases and parasympathomimetics tried had a much greater effect on the oral than on the bronchial fluid flow.

3. In poisoning with anticholinesterase fluid flow rates increased in parallel with the severity of the general signs of the poisoning.

References

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