GLYCOPYRROLATE METHOBROMIDE*

1. Effect on Salivary Secretion

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GLYCOPYRROLATE (Robinul®) was introduced in 1961 as a long-acting anti-cholinergic drug. Sun¹ and Moeller² reported the first clinical studies on its ability to control gastric acidity. All subsequent papers on glycopyrrolate continued to be related to gastroenterology until 1970 when Boatright and associates³ reported on its pre-operative use to forestall the consequences of the aspiration of acid gastric juice during tonsillectomy. Because of the conspicuous absence of side effects revealed in many of these studies, it was logical that glycopyrrolate should commend itself for the study of applications more directly of interest to the anaesthetist.

Glycopyrrolate is a quaternary ammonium compound with the following structural formula:

(1-methyl-3-pyrrolidyl -phenyl-cyclopentane glycolate methobromide)

It is a white crystal with a molecular weight of 398.34 and a melting point between 193.2–194.5° C. Each ml of the injectable form contains:

Glycopyrrolate	0.2 mg
Water for Injection U.S.P.	q.s.
Chlorobutanol (preservative)	0.5 per cent

Potential uses of glycopyrrolate include pre-operative medication to reduce salivary secretion, administration in conjunction with neostigmine to reverse residual curarization, and finally the correction of vagus-induced bradycardia in the course of anaesthesia. Of these Ramamurthy and associates reported in 1971⁴ on the protection afforded by glycopyrrolate against neostigmine-induced bradycardia and they were impressed by the fact that less tachycardia resulted when glycopyrrolate was used for this purpose in place of atropine. This cardiovascular stability was later confirmed under the same circumstances by Klingemaier *et al.*⁵

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FIGURE 1. Pump and traps for the collection of saliva.

The study here reported was intended to (1) elucidate the efficacy of glycopyrrolate as an antisialogogue comparing two different doses of it with atropine and with a placebo, and (2) to determine the duration of action of glycopyrrolate as a drying agent and compare it to that of atropine.

A comprehensive study of many other properties of glycopyrrolate of interest to the anaesthetist is presently underway in our Department and will be reported at a later date.

METHOD

The method employed to test the saliva-inhibiting properties of glycopyrrolate was the same as that previously reported by us.⁶ Briefly, saliva is collected from one parotid duct by means of a suction cup device connected to a Stedman pump with collecting volume-calibrated traps interposed between suction cup and pump (Figure 1). Ten minutes after administration of the anticholinergic, salivary secretion is stimulated by the administration over a three-minute period of 3 ml of a mixture containing carbamylcholine chloride (Carbachol®) 0.004 per cent (0.12 mgm) and epinephrine 0.002 per cent (0.06 mgm). The effect of the saliva-stimulating mixture is recorded in ml of saliva secreted over a period of time. The assumption is made that the parotid response to stimulation is a measure of total salivary secretion. All drugs were administered intravenously.

Our first series of experiments was conducted on twelve volunteers, each being

submitted to four separate tests at weekly intervals using normal saline, atropine sulphate 0.4 mgm, glycopyrrolate 0.1 mgm or 0.2 mgm in a random sequence. At least one week was allowed to elapse between each test and care was taken to use the parotid gland on the same side for each test. All tests were carried out in the early to mid-afternoon and the individuals were neither restricted nor encouraged in their fluid intake, so that one may assume normal hydration. Pulse rate and electrocardiogram were monitored and recorded throughout the experiment.

When the data were reviewed it was found that glycopyrrolate 0.2 mgm had never been administered on the first occasion. Given the randomization of drug sequence this was fortuitous and the bias thus introduced would appear to be minimal.

The second series of tests was aimed at establishing the duration of action of glycopyrrolate. It was carried out on six volunteers who, again at random sequence, were given on one occasion the carbamylcholine-epinephrine solution to serve as control and on two other occasions either atropine or glycopyrrolate followed within ten minutes by the intravenous injection of the saliva stimulator, in much the same way as in the preceding portion of the study just described, except that sampling of saliva was limited to five minutes after cessation of the injection. Thereafter the subjects were brought back at varying periods of time when 3 ml of the stimulating solution was again injected over a period of three minutes and the amount of saliva secreted measured again over a period of five minutes. The total amount of saliva secreted after either antisialogogue over the five minute period could then be compared with the control and expressed as a percentage of control. Because of the unpleasant subjective sensations associated with the injection of carbamylcholine-epinephrine and also because of fear of exhausting the parotid gland and thus influencing the results, serial determinations were not carried out on the same individual; rather each volunteer was brought back after a specific but different time interval had elapsed from the original injection.

RESULTS

Cumulative volumes of saliva following the administration of glycopyrrolate 0.1 mgm, 0.2 mgm, atropine sulphate 0.4 mgm and normal saline were plotted separately for the twelve individual tests. An immediate striking observation was the marked difference in the volumes of saliva secreted from the parotid glands of different individuals. This was not a function of hydration as is evident from the fact that the volumes secreted after administration of the three test drugs bore a relation to the controls; where the saline experiment showed a relatively scant secretion of saliva, the volumes after the antisialogogues also were of a low order whereas, in cases of high control values, salivation after 0.1 mgm of glycopyrrolate was also relatively substantial. The two extremes are represented by subjects 4 (Figure 2) and 12 (Figure 3) and two more average examples by subjects 1 and 11 (Figures 4 and 5). Further, salivary secretion started from as early as 40 seconds after beginning of the injection in some individuals to some time after the injection had been completed in others. However, on the average,

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FIGURE 2. Antisialogogue effects of glycopyrrolate, and glycopyrrolate and atropine compared with control (saliva) in subject No. 4.

between 73 per cent and 83 per cent of the 30-minute total was secreted within five minutes after completion of stimulant administration.

Another observation which was made not infrequently was that secretion in some individuals tended to occur in spurts while in others it was a constant flow. Figure 2 shows a good example of such a spurt at the 20- to 30-minute level and Figure 3 shows temporary cessation of secretion between three and ten minutes. Related results consisting of the mean volumes of saliva secreted per minute from the previous observation are shown in Table I and are graphically presented in Figure 6.

Most of the saliva eventually secreted was produced within the first five minutes from the end of the administration of the stimulant and the rate of salivary secretion changed rather dramatically after five minutes (Figure 6). These complementary observations might be interpreted as suggesting that the volumes secreted from the beginning of stimulant administration through five minutes after completion of stimulant administration are likely to be the most reliable indicator of drug effects and that thereafter either "saliva stores" had become exhausted or the effect of the stimulant was subsiding. It would seem reasonable, therefore, to limit more detailed analysis to the observations of the first eight minutes of saliva collection following the beginning of the injection of the stimulant.

The relevant data for this period of observation for each of the twelve subjects are given in Table II; the means of the volumes for each of the four drug treatments are listed in Table III together with the appropriate standard deviations. For purposes of stabilizing variances, the statistical computations have been performed on the log-transformed observations, namely $\text{Ln}(\text{volume} + 1)$, where

FIGURE 3. Greatest difference in antisialogogue effects in subject No. 12.

Ln denotes the natural logarithm. The means of these transformed observations are shown in Table III. The analysis of variance performed on these transformed eight-minute volumes is summarized in Table IV and indicates that significant differences exist among the four treatment means. Comparisons of these four means by the Tukey W-procedure at the 1 per cent level (an experiment-wise error rate) indicate the following:

FIGURE 4. Salivary secretion subject No. 1 (see text).

FIGURE 5. Salivary secretion subject No. 11 (see text).

TABLE I
MEAN VOLUMES OF SALIVA SECRETED PER MINUTE (ml/min) SINCE PREVIOUS OBSERVATION. THE MEANS ASSOCIATED WITH t = 0 ARE
BASED ON TIME-INTERVAL OF THREE MINUTES

Treatment	Time after Administration of Stimulant									
	0	1 min	2 min	3 min	4 min	5 min	10 min	15 min	20 min	30 min
Saline	0.893	1.575	1.104	0.588	0.296	0.208	0.076	0.053	0.049	0.044
Glycopyrrolate 0.1 mg	0.134	0.560	0.302	0.208	0.096	0.129	0.055	0.018	0.011	0.019
Atropine 0.4 mg	0.034	0.063	0.042	0.038	0.017	0.021	0.009	0.005	0.002	0.003
Glycopyrrolate 0.2 mg	0.006	0.017	0.022	0.045	0.017	0.021	0.003	0.003	0.001	0.000

FIGURE 6. Graphic representation of the mean volumes of saliva secreted per minute as tabulated in Table I.

1. The mean of the saline 1 ml treatment is significantly greater than each of the means of the other three drug treatments.
2. The mean of the glycopyrrolate 0.1 mg treatment is significantly greater than the means of both the atropine 0.4 mg and glycopyrrolate 0.2 mg treatments.
3. A significant difference is not detected between the atropine 0.4 mg and glycopyrrolate 0.2 mg treatments.

As stated previously the electrocardiogram was continuously monitored

TABLE II

VOLUME OF SALIVA (ml) SECRETED FROM BEGINNING OF ADMINISTRATION OF CARBACHOL/EPINEPHRINE TO FIVE MINUTES AFTER COMPLETION OF ADMINISTRATION

Subject	Saline (1 ml)	Atropine (0.4 mg)	Glycopyrrolate (0.1 mg)	Glycopyrrolate (0.2 mg)
1	4.4	0.2	0.9	0.1
2	4.5	0	4.3	0.05
3	5.2	0.5	2.7	0.3
4	2.05	0	0.25	0
5	11.15	1.0	0.60	0.6
6	5.1	0.5	0.8	0
7	5.7	0	1.2	0
8	6.9	0.65	1.05	0.3
9	8.0	0.3	1.8	0
10	5.1	0.05	3.2	0.08
11	2.7	0.0	0.3	0.03
12	16.6	0.3	3.4	0.2

TABLE III

MEAN OF VOLUMES OF SALIVA (ml) FOR OBSERVATIONS IN TABLE II AND FOR LOG-TRANSFORMED OBSERVATIONS. STANDARD DEVIATIONS ARE IN PARENTHESES

	Salivary Volume (ml)	Ln (Volume +1)
Saline	6.4500 (3.988)	1.899
Atropine (0.4 mg)	0.2915 (0.320)	0.230
Glycopyrrolate (0.1 mg)	1.7083 (1.359)	0.884
Glycopyrrolate (0.2 mg)	0.1383 (0.183)	0.119

TABLE IV

TABLE OF ANALYSIS OF VARIANCE APPLIED TO LOG-TRANSFORMED OBSERVATIONS

Source	SS	DF	MS	F
Subjects	2.6980	11	0.2453	
Treatments	24.0273	3	8.0091	80.6719 (p < 0.001)
Residual	3.2765	33	0.0993	
Total	30.0019	47		

SS: Sums of squares. DF: Degrees of freedom. MS: Mean square. F: Mean squares for treatment/residual.

throughout all experiments and inspection of recordings failed to reveal any changes in the complexes in any of the runs.

Pulse rate changes are presented in Tables V and VI. The one shows the incidence of changes during the first five minutes after completion of the administration of the anticholinergic, and the other lists changes in pulse rate during the three minutes of administration of the carbamylcholine-epinephrine mixture. In neither case are incidence nor extent of alteration significant.

The results obtained in the second part of the study are illustrated in Figure 7. In none of the individuals was any significant salivary secretion obtained after glycopyrrolate within the first seven hours from injection of this agent and only at the ten-hour study was saliva again secreted under the influence of the stimulant. On the other hand, while no saliva was obtained with atropine at the

TABLE V
INCIDENCE OF PULSE RATE CHANGES AT FIVE MINUTES AFTER
ADMINISTRATION OF ANTICHOLINERGIC
(BASELINE IS ADMINISTRATION OF ANTICHOLINERGIC)

Pulse rate change (beats per min)	Saline	Atropine	Glycopyrrolate	
			0.1	0.2
Increase 10 or more	1	2	0	2
Change less than ± 10	11	7	9	10
Decrease 10 or more	0	3	3	0

TABLE VI
INCIDENCE OF PULSE RATE CHANGES OVER THE THREE-MINUTE
ADMINISTRATION OF STIMULANT

Pulse rate change (beats per min)	Saline	Atropine	Glycopyrrolate	
			0.1	0.2
Increase 10 or more	7	6	8	7
Change less than ± 10	2	3	2	3
Decrease 10 or more	0	1	0	1
Not Stated	3	2	2	1

FIGURE 7. Measurements of saliva secretion in the 10-hour period after injection of glycopyrrolate expressed as a percentage of control. ●, Atropine; ○, Glycopyrrolate.

two hour observation, partial salivary activity had returned at three hours and at the ten hour test was twice that of glycopyrrolate. The three hour observation is interesting in that the value for atropine obtained is higher than that at either four, five or seven hours. This can best be explained as an individual variation and is supported by the fact that in this particular person even with glycopyrrolate a minimal amount of saliva (1 per cent of control) was obtained. It would appear, therefore, that this particular person was somewhat more resistant to the effect of either drying agent than all others. It is also interesting to note that there was little difference between the four-, five- and seven-hour results after atropine which might indicate that the return of activity is not linear but rather occurs in a step-wise fashion. Be this as it may, the much longer duration of glycopyrrolate as a drying agent is striking and reproducible, as is the shorter duration of atropine. These findings correlate well with the subjective reports from our subjects that there was intense dryness for more than seven hours after injection of glycopyrrolate to the point of pharyngeal soreness and that at all times, quite apart from duration, the sensation of dryness after glycopyrrolate was much more intense than after atropine.

CONCLUSIONS

From the results of this study, one can reasonably draw the following conclusions:

1. Both glycopyrrolate at doses 0.1 mgm and 0.2 mgm and atropine at dose of 0.4 mgm have significant antisialic effect.
2. Atropine 0.4 mgm and glycopyrrolate 0.2 mgm are virtually indistinguishable with respect to the magnitude of antisialic effect.
3. The antisialogogue action of glycopyrrolate far exceeds that of atropine in the doses studied, the ratio being in the region of 3:1.

Further, the results of this study support the hypothesis that the magnitude of the antisialic effect of glycopyrrolate is dose-related. Subjectively, the drying effect of glycopyrrolate is very much more intense than that of atropine.

RÉSUMÉ

Les effets du Glycopyrrolate sur les sécrétions salivaires ont été étudiés et comparés à ceux de l'Atropine et à ceux d'un placebo chez un groupe de volontaires.

On a observé le volume de sécrétions salivaires provoquées par un mélange de Chlorure de Carbamylcholine et d'Epinephrine après que l'on eut administré un antisialogogue (Atropine, Glycopyrrolate ou Soluté Physiologique).

Cette étude a d'abord établi qu'il n'y a pas de différence significative au point de vue suppression des sécrétions salivaires entre une dose de 0.4 mg d'Atropine et 0.2 mg de Glycopyrrolate. A la dose de 0.1 mg, le Glycopyrrolate est moins efficace mais supprime encore plus les sécrétions que le placebo.

Les fréquences cardiaques n'ont pas été modifiées de façon significative après administration d'Atropine ou de Glycopyrrolate.

Une seconde phase de l'étude a établi clairement que l'effet du Glycopyrrolate dure au moins trois fois plus longtemps que celui de l'Atropine.

De plus tous les volontaires ont été unanimes à affirmer que l'état de sécheresse était beaucoup plus marqué après Glycopyrrolate qu'avec l'Atropine.

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