The Effect of Neostigmine, Atropine and Ephedrine on Heart Rate in Man

J. S. Gravenstein, M.D., and Haven M. Perkins, M.D.†*

The effects of different doses of neostigmine methylsulfate, atropine sulfate and ephedrine on the heart rate in fit volunteers were studied. Neostigmine decreased heart rate equally in unmedicated subjects and in subjects premedicated with 25 mg. ephedrine, but decreased the rate much more in subjects premedicated with 0.6 to 1.5 mg. atropine sulfate. Under the experimental conditions, from sevenfold to twentyfold doses of atropine sulfate were required to block completely the effect of neostigmine methylsulfate on heart rate. About twice the weight of atropine sulfate was required to prevent neostigmine methylsulfate from lowering heart rates below base line. The effect of neostigmine on heart rate began within a minute after intravenous injection, reached a maximum in about 20 minutes, and lasted for more than an hour.

CLINICAL practice dictates that atropine sulfate be given before or with neostigmine methylsulfate, when the latter is employed to combat muscle paralysis produced by nondepolarizing muscle relaxants, or when testing patients with suspected myasthenia gravis. Atropine is administered in these instances to decrease or abolish the effects of neostigmine on the smooth muscles of the gastrointestinal tract and bronchi, on mucous glands and on the heart. Clinical experience suggests that about one-half weight unit of atropine sulfate suffices to block the undesirable effects of one weight unit of neostigmine methylsulfate. We were unable to find data in the literature to confirm this clinical impression and, therefore, studied the effect of atropine and neostigmine on heart rate. For comparison with atropine we included ephedrine in our experiments since this sympathomimetic amine has a positive chronotropic effect [1] and, furthermore, is used with neostigmine in the treatment of myasthenia gravis.

* Professor and Chairman, Department of Anesthesiology, College of Medicine, University of Florida, Gainesville, Florida.

† Assistant Professor, Department of Anesthesiology, College of Medicine, University of Florida, Gainesville, Florida.

Accepted for publication January 11, 1966.

Methods

Sixteen healthy male students served as subjects. An intravenous infusion of 5 per cent dextrose in water was started to allow subsequent intravenous injection of drugs without disturbing the subject. The students rested for 30 minutes before and remained in the supine position throughout the experiment. The pulse rate obtained from an ECG was always counted for a full minute; reported values refer to beats per minute. For statistical analyses linear least square analyses were performed.[2] The drugs employed were commercially available neostigmine methylsulfate (Prostigmine methylsulfate, Hoffmann-LaRoche), atropine sulfate (Burroughs Wellcome) and ephedrine (Burroughs Wellcome). Weights of neostigmine and atropine were calculated as the salts. All drugs were injected rapidly.

Two experiments were performed:

Experiment 1. On one day, a group of 7 students was given atropine 0.6 mg., and after 10 minutes, neostigmine 0.03, 0.1, 0.3 and 0.4 mg. at 10-minute intervals. On another day the four doses of neostigmine were given first, followed by atropine 10 minutes after the fourth dose. Three of the 7 students had the atropine-neostigmine sequence first and the reverse sequence on another day, whereas the other subjects received the sequence in opposite order.

An identical experiment was done in a different group of 5 students, but here ephedrine 25 mg. was substituted for atropine. Again the sequence was alternated.

Experiment 2. Groups of 3 subjects were given a dose of atropine and 10 minutes later a dose of neostigmine. The following combinations were tested:

Atropine (*mg.*)	Neostigmine (*mg.*)
0.6	0.83
0.8	0.83
1.0	0.83
1.5	0.83
1.5	0.4
1.5	0.2
1.5	Normal saline

Results

Experiment 1. The effects of graded doses of neostigmine on heart rate in unmedicated subjects and after medication with atropine or ephedrine are plotted in figure 1. We assumed here that the neostigmine doses were cumulative and entered these cumulative doses on a logarithmic scale. After the first dose of neostigmine, 0.03 mg., heart rate slowed. The first three doses of neostigmine were about equally effective, whether given to unmedicated subjects or to subjects premedicated with 25 mg. ephedrine, even though ephedrine had raised mean rates within 10 minutes, from 67 to 76. Atropine 0.6 mg. had raised heart rates from 61 to 76, but here neostigmine had a different action: the first dose was ineffective and subsequent larger doses more effective in slowing rates than was the case in unmedicated subjects or those premedicated with ephedrine.

This strikingly stronger effect of neostigmine in subjects pretreated with atropine (as compared to ephedrine or no premedication) deserved careful analysis utilizing all data.

The differences were therefore assessed by means of least square analyses of variance. In table 1, the data are summarized on the left in terms of mean values and on the right the regression coefficients (slopes) are shown. The analyses were based upon deviations from the predrug control values. In order to increase precision, the data from the two groups of unmedicated subjects receiving neostigmine were pooled. The means shown on the left of table 1 represent mean values for the time periods indicated in each comparison. All data are graphically presented in figure 2,

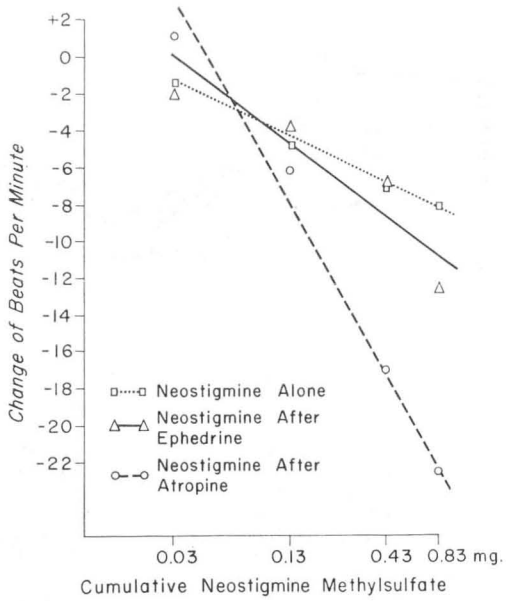

FIG. 1. Mean differences between the heart rates counted immediately prior to the first dose of neostigmine and 10 minutes after each dose of neostigmine are shown. The abscissa gives cumulative values of four doses given in 10-minute intervals. Neostigmine alone was given to 12 subjects, neostigmine after ephedrine to 5 and after atropine to 7. Straight lines were fitted by approximations.[2]

which should be consulted together with the statistical analyses shown in table 1.

Four comparisons were made: In the first comparison (1–10 minutes), the effects of 0.03 mg. neostigmine given alone (P), given 10 minutes after 0.6 mg atropine (AP), or 10 minutes after 25 mg. ephedrine (EP) were analyzed. After neostigmine 0.03 mg. was given to unmedicated subjects, mean heart rates slowed slightly but consistently. Here neostigmine appeared significantly more effective in the ephedrine than in the atropine group (where 0.03 mg. neostigmine had no effect). The positive mean differences in the table indicate that heart rates in subjects premedicated with ephedrine (EP) or atropine (AP) were still significantly elevated above predrug control values. Since both pretreated groups had mean rates of about 76 beats the significant differences of 6.67 between atropine and ephedrine (AP-EP) indicates that the predrug control values in the atropine group were by chance about 6 beats lower than the re-

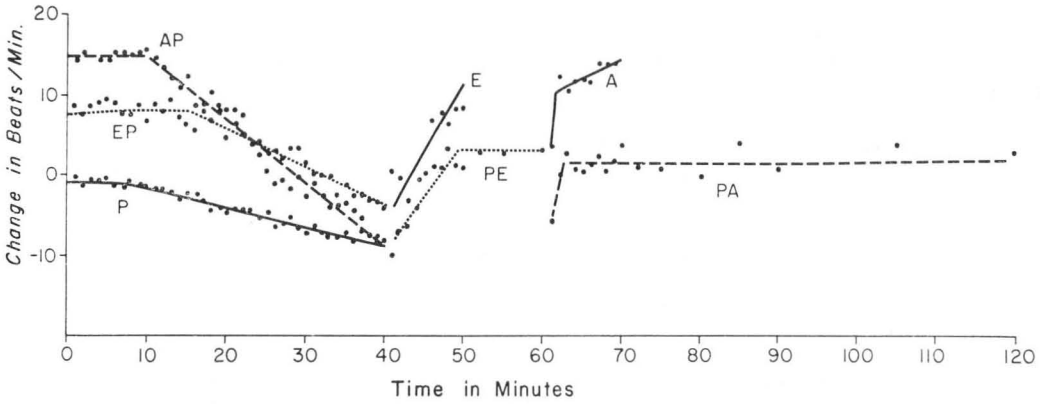

FIG. 2. Deviations from predrug control heart rates. Each point represents the mean deviation of heart rates from 5 (E, EP, PE), 7 (A, AP, PA) or 12 (P) subjects. The lines are the slopes fitted for the means (table 1). AP shows the effect of four doses of neostigmine on heart rates elevated by atropine given 10 minutes before time 0. At times 0, 10, 20 and 30 neostigmine 0.03, 0.1, 0.3 and 0.4 mg., respectively, were injected. EP shows data similarly obtained after premedication with ephedrine. The data labeled P were obtained from 12 subjects (P) who were given 25 mg. ephedrine at minute 40 (PE). For comparison data from the same 5 subjects receiving ephedrine alone (E) at a different time is also entered under minutes 40–50. Seven of the 12 subjects premedicated with neostigmine were observed for 30 minutes and then (at minute 60 in the figure) given 0.6 mg. atropine (PA). For comparison data from the same subjects treated at a different time with atropine alone were entered at minute 60. See table 1 for statistical analysis.

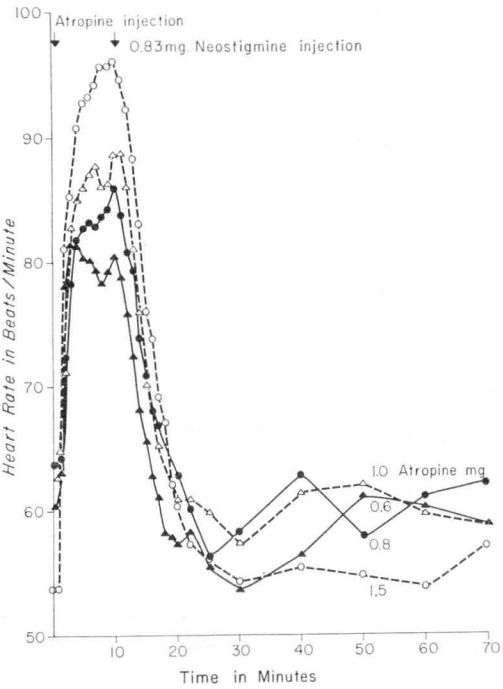

FIG. 3. Mean heart rates from a group of 3 subjects studied four times are shown. Once the subjects were given 0.6, once 0.8, and once 1.0, and 1.5 mg. of atropine sulfate intravenously, and 10 minutes later 0.83 mg. neostigmine.

spective values in the ephedrine groups (61 versus 67).

In the second comparison (11–40 minutes) the effects of the subsequent three doses of neostigmine (0.1, 0.3 and 0.4 mg.) were pooled. In this period neostigmine alone (P) lowered mean rates by about five beats below predrug control rates, but was not successful in bringing below base line the mean rates of the subjects pretreated with atropine (AP) or ephedrine (EP). However, the mean rates in the atropine group were now lower and the slope significantly steeper than in the ephedrine group (AP-EP). The ephedrine group showed a steeper slope than the neostigmine group (EP-P).

In the third comparison, the effect of 25 mg. ephedrine alone and 25 mg. ephedrine given 10 minutes after the last neostigmine dose are shown. The slopes of heart rates in response to ephedrine were not significantly different whether or not the volunteers had received neostigmine previously, even though mean (and peak) values reached with ephedrine were significantly lower in the group pretreated with neostigmine.

The subjects given atropine after neostigmine

TABLE 1. Response to Drug Combinations

Comparison	*Mean*	*SE (mean)*	*t*	*Slope*	*SE (slope)*	*t*
(1) Effect of 0.03 mg. neostigmine (P) alone and after atropine (A) or ephedrine (E) (1–10 minutes)						
AP	14.99	0.539	27.75†			
EP	8.32	0.512	16.31†			
P	−1.00	0.162	6.25†			
AP-EP	6.67	2.648	2.52*			
(2) Effect of subsequent neostigmine (P) doses alone and after atropine (A) or ephedrine (E) (11–40 minutes)						
AP	2.45	0.412	5.97†	−0.793	0.048	16.52†
EP	3.14	0.485	6.54†	−0.465	0.056	8.30†
P	−5.35	0.195	27.44†	−0.225	0.023	9.78†
AP-EP	−0.69	0.202	3.42†	−0.328	0.074	4.43†
EP-P	8.49	0.434	19.56†	−0.240	0.050	4.80†
(3) Effect of ephedrine alone and after total of 0.83 mg. neostigmine (41–50 minutes)						
PE	−2.14	0.728	2.94*	1.395	0.253	5.51†
E	3.74	0.425	8.80†	1.744	0.148	11.78†
PE-E	−5.88	0.852	6.90†	−0.349	0.293	1.19
(4) Effect of atropine alone and after total of 0.83 mg. neostigmine (after 61 minutes)						
PA	1.78	0.467	3.81†	0.021	0.026	0.81
A	12.89	0.743	17.35†	0.551	0.324	1.70
PA-A	−11.11	0.8297	13.39†	−0.530	0.291	1.82

* Significant at 5 per cent level.
† Significant at 1 per cent level.
See text for explanation and compare with figure 2.

were observed for 30 minutes after the fourth dose of neostigmine before atropine was given. The fourth comparison shows the effect of atropine after neostigmine (61 minutes) and of atropine given to unmedicated subjects. Premedication with neostigmine 30 minutes earlier reduced the response to atropine significantly. The difference between slopes (PA-A; $t = 1.82$; $0.2 > P$ [2 tail] > 0.1) may have arisen by chance.

In figure 2 the data from patients receiving neostigmine after 0.6 mg. atropine show that heart rates returned to base line at 25 minutes, after a total of 0.43 mg. neostigmine had been given. In order to determine doses for atropine and neostigmine which would cancel their opposing effects on heart rate the second experiment was done.

Experiment 2. Groups of 3 subjects were given increasing doses of atropine, and 10 minutes later a standard dose of 0.83 mg neostigmine (fig. 3). Since this did not provide the desired ratio of neostigmine to atropine, we gave a standard dose of 1.5 mg. atropine and 10 minutes later, normal saline or decreasing doses of neostigmine (fig. 4).

The ineffectiveness of 0.6 to 1.5 mg. atropine in abolishing the action of 0.2 to 0.83 mg. neostigmine becomes apparent in figures 3 and 4. In these figures, it can be seen that 0.83 mg. neostigmine brought rates back to base line even after 1.5 mg. atropine and lowered rates below base line when smaller doses of atropine were given as pretreatment (fig. 3). As little as 0.2 mg. neostigmine given 10 minutes after 1.5 mg. atropine failed to bring heart rates back to the pre-atropine base line although this dose of neostigmine significantly slowed rates elevated by a dose of atropine seven times greater.

Figures 3 and 4 also show that the neostigmine effect began in the first minute, reached a maximum after 20 minutes, and

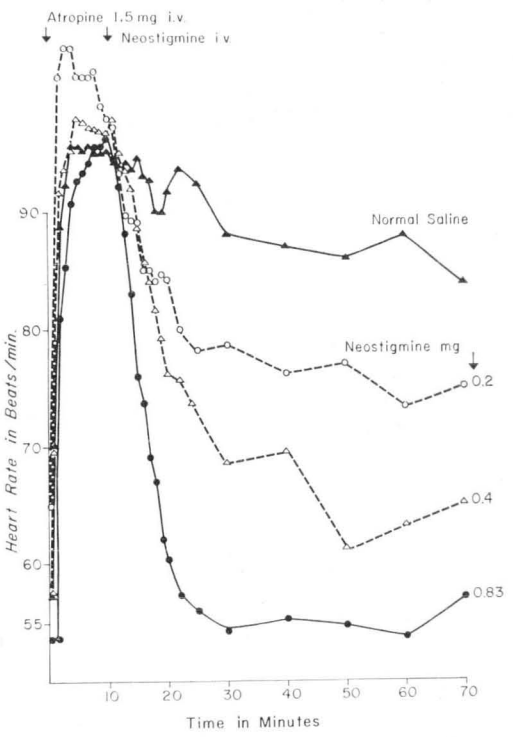

FIG. 4. Four groups of 3 subjects were given 1.5 mg. atropine intravenously and 10 minutes later on one occasion an injection of normal saline and on other occasions 0.2, 0.4 or 0.83 mg. neostigmine. Mean heart rates are shown. Data from the group receiving 0.83 mg. neostigmine are also shown in figure 3.

lasted more than one hour after an intravenous injection.

We found no cardiac arrhythmias with any dose of neostigmine, atropine, or ephedrine.

Discussion

The data suggest that as little as 0.03 mg. neostigmine given to subjects weighing 75 to 80 kg. might have lowered heart rate slightly (figs. 1 and 2, table 1). It is conceivable that bias was responsible for this observation and that only the larger doses of neostigmine were active. Such bias could be introduced by a spontaneous slowing of heart rates in the resting, unmedicated subjects and by decay of the positive inotropic effect of ephedrine in the students thus premedicated. A decay of the ephedrine effect may have contributed to the seemingly large response to the last neostigmine dose injected 40 minutes after ephedrine had been given (fig. 1).

The response of heart rates to neostigmine in the subjects premedicated with atropine differed significantly from that of the unpremedicated subjects and that of the subjects premedicated with ephedrine. In the atropine experiment 0.03 mg. neostigmine was ineffective, but unexpected effective results were seen with subsequent larger doses of neostigmine. The lack of an effect of the smallest neostigmine dose may again mean merely that this was an ineffective dose under any circumstances or that here atropine (in a dose 20 times larger than that of neostigmine) proved to be an effective anticholinergic substance. Atropine was, however, quite ineffective in preventing the action of larger doses of neostigmine. This was not because the atropine effect decayed so rapidly. For normal duration of atropine effect, see saline after atropine (fig. 4), and the sustained slightly elevated rates in the experiment where atropine was given after neostigmine (fig. 2 and table 1), as well as our earlier report on atropine.[3] It seems indeed that the heart rates quickened by atropine were particularly sensitive to slowing by the second, third and fourth doses of neostigmine in the first experiment, or by all neostigmine doses in the second experiment. This assumption is further supported by the observation that neostigmine lowered heart rates to significantly lower levels after atropine than after ephedrine, not only where rates are expressed in terms of decrease from peak values but also in terms of deviation from resting predrug control values.

The differences in dose response slopes (fig. 1 and table 1) suggest that neostigmine triggered a process in subjects premedicated with atropine which did not operate in unmedicated volunteers or in those pretreated with ephedrine. This process must have involved an interaction between atropine and neostigmine. We suggest that the larger doses of neostigmine made enough acetylcholine available to compete successfully with atropine for receptors involved in regulation of heart rate. While there is no unanimity of evidence,[4] it is possible that atropine is a simple competitive antagonist to acetylcholine.[5] Data collected

n man in whom many compensatory reflexes are at play, and studies using neostigmine without measurements of acetylcholine concentrations in the target organ, should not be marshalled to support the thesis that atropine s a competitive inhibitor. Nevertheless, our data would support rather than contradict the existence of such a mechanism.

The demonstration that atropine was a relatively poor agent to counteract the negative chronotropic effects of neostigmine does not mply that atropine should be abandoned in the preparation for injection of neostigmine n curarized patients. If atropine in the dosages commonly used prior to neostigmine is really as effective in preventing cardiovascular side-effects of neostigmine as generally believed, it may be acting on different sites, or may be effective by reducing or modifying rather than abolishing such a neostigmine effect. The interaction of neostigmine and atropine during anesthesia may be markedly affected by the presence of anesthetic agents. However, in the recovery room, bradycardia s not infrequently observed in patients treated with 2 to 3 mg. neostigmine after 0.5 to 1 mg. atropine.

The demonstration that ephedrine is a relatively effective means of counteracting the negative chronotropic effect of neostigmine does not necessarily imply that ephedrine should be used with neostigmine. While ephedrine with neostigmine is used in some patients with myasthenia, the use of ephedrine in anesthetic procedure with neostigmine should be carefully evaluated because a simultaneous increase of sympathetic activity and parasympathetic tone may lead to disturbances in cardiac rhythm.

This work was supported in part by National Institutes of Health Grant HE-07129. Dr. D. Van Der Reyden helped with statistical computations.

References

1. Andersen, T. W., and Gravenstein, J. S.: Mephentermine and ephedrine in man, Clin. Pharmacol. Ther. **5**: 281, 1964.
2. Hoffmann, R. G.: Statistics for Medical Students. Springfield, Ill., Charles C Thomas, 1963.
3. Gravenstein, J. S., Andersen, T. W., and DePadua, C. B.: Effects of atropine and scopolamine on the cardiovascular system in man, ANESTHESIOLOGY **25**: 123, 1964.
4. Clark, A. J.: The antagonism of acetylcholine by atropine, J. Physiol. (London) **61**: 547, 1926.
5. Arunlakshana, O., and Schild, H. O.: Some quantitative uses of drug antagonists, Brit. J. Pharmacol. **14**: 48, 1959.

ANESTHESIOLOGY CENTER Started in 1950, the Anesthesiology Center Copenhagen is maintained by the World Health Organization, the National Health Service of Denmark and the Medical Faculty of the University of Copenhagen. Physicians from countries with insufficient training facilities in anesthesia who have been in anesthesia for at least two years, are given stipends by the WHO. Refresher courses are held every two years. Eight teaching hospitals are used. The faculty consists of about 30 persons in anesthesia and related fields. Prominent guest lecturers are also invited. The trainees take a final examination; successful candidates receive the title "D.A." (Diploma Anesthesiologica) from the University of Copenhagen. This examination is difficult and only 50 to 55 per cent of the candidates pass it. This is contrasted with the conditions in Germany where certification as a specialist is granted after completion of the required training program without any formal examination. Higher standards and stature for anesthesia would be derived from a more rigid theoretical and practical training. *(Nolte, H.: The Anesthesiology Center Copenhagen, Der Anaesthesist 14: 312 (Oct.) 1965.)*