The Parasympathomimetic Activity of Atropine and Atropine Methylbromide

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Atropine in small doses decelerates heart rate. This parasympathomimetic effect, which has been attributed to a central vagal stimulating action of atropine sulfate, was studied in dogs by measuring heart rate and P–R intervals. The action of atropine sulfate (which has access to the brain) was compared to that of its quaternary derivative, atropine methylbromide, which is believed not to cross the blood–brain barrier. Atropine methylbromide was more potent than atropine sulfate in accelerating heart rate. In anesthetized dogs, but not in dogs anesthetized with chloralose, atropine sulfate and atropine methylbromide in small doses slowed heart rate. In awake and anesthetized dogs both drugs prolonged P–R intervals. Bilateral cervical vagotomy abolished the drug effect on the P–R interval, but when the distal limb of the vagus was stimulated electrically both atropine sulfate and atropine methylbromide again prolonged the P–R interval. We infer that atropine methylbromide and atropine sulfate exerted peripheral parasympathomimetic effects on the heart. The possibility that atropine sulfate has central vagal stimulating effect was not excluded.

The usual anticholinergic effect of atropine is elevation of heart rate. In conscious man, small intravenous doses of atropine sulfate cause bradycardia,4,2 apparently through a cholinergic or parasympathomimetic effect of the drug. Earlier studies5–6 attributed this bradycardia to a stimulating effect of atropine on vagal centers. Other experimental and clinical studies indicated that atropine may also cause changes in conduction within the heart.7–8,9 It is not known whether atropine's parasympathomimetic and direct effects on the heart interact with those of neostigmine to cause untoward results such as cardiac arrest.8–12 In conscious volunteers a more pronounced slowing occurs with neostigmine if the subjects are pretreated with atropine than if neostigmine is given alone.13

To study the apparent parasympathomimetic activity of atropine we recorded changes in heart rate and P–R interval in the dog after incremental doses of atropine sulfate. If the parasympathomimetic effects of atropine were centrally mediated, they should not occur after atropine methylbromide. This methyl derivative is a charged molecule, and is not believed to cross the blood–brain barrier readily, hence the action is primarily in the periphery.14 Atropine sulfate does cross the blood–brain barrier easily, and may act centrally as well as peripherally. Our hypothesis was that all parasympathomimetic effects are centrally evoked and that the quaternary derivative, lacking ready access to the brain, is therefore devoid of parasympathomimetic effects.

Since we were primarily interested in the parasympathomimetic effects of the atropine salts, we decided to pretreat some animals with neostigmine to heighten those atropine effects that involve acetylcholine as a transmitter. We postulated that neostigmine would affect the actions of atropine differently than those of atropine methylbromide because the former but not the latter has central vagal stimulating effects.

Methods

We used nine mongrel dogs of either sex weighing from 11 to 23 kg. A large indwelling venous catheter was placed in a foreleg vein for drug administration. Because of undesirable effects of neostigmine on the gut of

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the conscious dog, all dogs scheduled for neostigmine premedication were anesthetized. After anesthesia was induced with 75 to 100 mg/kg alpha-chloralose, a cuffed endotracheal tube was placed. Respiration was controlled with a Harvard piston ventilator, and body temperature was maintained within normal range with heating pads. ECG skin electrodes were put on each extremity, and the electrocardiogram recorded continuously on a Sanborn recorder.

In anesthetized dogs, either atropine sulfate or atropine methylbromide with or without premedication with neostigmine (for comparison) was given the same animals in repeated experiments according to a balanced design that allowed at least two days' rest between experiments and provided for different drug sequences. Neostigmine was injected intravenously in a dose of 0.25 mg five minutes before the first dose of atropine sulfate or atropine methylbromide. Neostigmine followed by several saline injections provided control values. The experiments were repeated, omitting neostigmine, in six unanesthetized dogs allowed to relax in a leg harness.

Thirteen to 15 doses of the belladonna drugs were given, beginning with about 0.0003 mg/kg to a cumulative dose approaching 1.0 mg/kg (Table 1). All drugs were injected rapidly intravenously in volumes of 2 ml, followed by 5 ml physiologic saline solution. Exactly three minutes elapsed between injections. Doses were calculated from molecular weight equivalents of atropine sulfate (mol weight = 694.82) which contains two atropine bases, and atropine methylbromide (mol weight = 384.29) which contains only one atropine base. Atropine sulfate and atropine methylbromide are racemic. Since two mols atropine methylbromide are equivalent in base to one mol atropine sulfate, the following formula was used to calculate equivalent doses:

\[
\frac{(1) \cdot \text{atropine sulfate (694.82)}}{(2) \cdot \text{atropine methylbromide (384.29)}} = x
\]

providing a conversion factor \( x = 1.10 \).

In the unanesthetized animals only 12 doses were given, for a total dose of 0.1 mg/kg atropine sulfate and 0.11 mg/kg atropine methylbromide.

Heart rates were counted for 15 seconds in 30-second intervals from the ECG recordings. At a paper speed of 100 mm/second, the P-R intervals were measured at 30-second intervals. For each three-minute period, heart rates and P-R intervals were averaged. Data were plotted on semilogarithmic scales (see figures), which obscured the fact that all doses were given at three-minute intervals. The dogs served as their own controls.

In another group of seven dogs anesthetized with chloralose, three animals were given atropine methylbromide and four, atropine sulfate. In these dogs, heart rate and P-R intervals were measured, followed by bilateral cervical vagotomy. Heart rate and P-R intervals were again recorded. A distal limb of the vagus was then electrically stimulated with current from a Grass nerve stimulator for 30 seconds (0.01 msec, 6-8 volts at 100 CPS) so that the rate and P-R intervals returned toward prevagotomy control values. The sequence of events in this experiment is shown in Table 2.

In order to show the influence of electrical stimulation of the distal vagus (expected to lengthen P-R intervals) on the atropine effect expected to shorten P-R intervals), the P-R intervals are expressed as differences according to the formula: \((9 - 7) - 4\), in which the numbers refer to the steps shown in Table 2.
This formula corrects the measurement of step 9 by excluding P-R interval changes without vagal stimulation after the atropine dose (step 7), and during vagal stimulation without atropine (step 4). After administration of small doses of atropine vagal stimulation slowed heart rate to control levels. When vagal stimulation slowed rates without reaching control rates, “partial vagal blockade” was said to exist. “Complete vagal blockade” existed when vagal stimulation was without effect on heart rates.

Standard errors of measurement were calculated and data were analyzed by Student’s t test.

Results

In unanesthetized dogs, atropine sulfate caused a slowing of five heart beats per minute, significantly different (P < 0.05) from a four-heart-beat per minute slowing caused by atropine methylbromide (fig. 1). Atropine methylbromide, however, was more potent, i.e., slowing occurred with lower doses. Significant slowing did not occur with any drug in anesthetized dogs. The slight slowing observed with atropine sulfate (fig. 3) after neostigmine in the anesthetized dogs might have occurred by chance (0.1 > P > 0.05).

Figures 2 and 3 show the changes in heart rates of anesthetized dogs receiving atropine or the quaternary methyl derivative. The dose-response curves for atropine sulfate and atropine methylbromide were parallel, but atropine methylbromide was more potent. Premedication with neostigmine shifted the dose–response curves of both drugs significantly (P < 0.05) to the right without significant slowing of rate following smaller doses of either atropine drug.

Chloralose abolished the bradycardia and shifted the left portion of the dose–response curves of heart rate with atropine sulfate and atropine methylbromide significantly to the left. To increase the rate by 25 beats per minute in the awake animal required about 0.015 mg/kg atropine sulfate and 0.0044 mg/kg atropine methylbromide, but under chloralose anesthesia, only 0.008 mg/kg and 0.0033 mg/kg, respectively, were needed (see figs. 1, 2 and 3). When rates had accelerated by 50 beats per minute or more, drug effects in awake and anesthetized animals were identical. Anesthesia had no significant effect on the dose–response curves of P-R intervals.

In awake and anesthetized animals, a significant (P < 0.05) prolongation of P-R interval was seen after both atropine and atropine methylbromide (figs. 4–7). Neostigmine did not significantly shift either the atropine or the atropine methylbromide dose–response curve for P-R intervals.

The changes in P-R intervals were not parallel to the changes in heart rates. Acceleration of heart rate in response to the belladonna drugs was well under way before a significant prolongation of P-R interval could be detected (figs. 4–7). The peak prolongation of P-R interval occurred long before peak heart rates were obtained. P-R intervals leveled off at values significantly lower than control, before heart rates had reached a peak. Neostigmine alone did not have a statistically significant effect on P-R interval.

During prolongation of P-R intervals, dropped beats and premature ventricular contractions occurred in several of the awake and anesthetized dogs, with and without neostigmine. These abnormalities disappeared when the P–R interval was again at baseline level, or reduced.

In vagotomized animals, neither atropine nor atropine methylbromide significantly affected P-R intervals as long as the distal vagus was not stimulated. In the presence of elec-

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**Table 2: Protocol for Experiments With Vagal Stimulation**

<table>
<thead>
<tr>
<th>Step</th>
<th>Time in Minutes</th>
<th>Procedure</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>Dog under anesthesia</td>
<td>Rate + P-R</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>Bilateral vagotomy</td>
<td>Rate + P-R</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>Electrical stimulation of distal vagal stump</td>
<td>Rate + P-R</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>Electrical stimulation off</td>
<td>Rate + P-R</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>Atropine dose one i.v.</td>
<td>Rate + P-R</td>
</tr>
<tr>
<td>6</td>
<td>+ 1.0</td>
<td>Electrical stimulation on</td>
<td>Rate + P-R</td>
</tr>
<tr>
<td>7</td>
<td>+ 1.5</td>
<td>Electrical stimulation on</td>
<td>Rate + P-R</td>
</tr>
<tr>
<td>8</td>
<td>+ 1.5</td>
<td>Electrical stimulus off</td>
<td>Rate + P-R</td>
</tr>
<tr>
<td>9</td>
<td>+ 3.0</td>
<td>Atropine dose two i.v.; repeat steps 7–11</td>
<td>Rate + P-R</td>
</tr>
</tbody>
</table>
In the conscious animal, low doses of both drugs slowed heart rate, i.e., both drugs exerted a parasympathomimetic effect. When chloralose anesthesia abolished the slowing effect, the left portion of the dose-response curves of both atropine sulfate and atropine methylbromide were shifted to the left. Since chloralose anesthesia did not affect the action of either drug on P–R interval it is suggested that the bradycardic effect of the belladonna drugs (chloralose sensitive) differed from the effect on the P–R interval (not chloralose sensitive). We have no explanation for the selective effect of chloralose anesthesia, and presumably other anesthetics, on abolition of the bradycardic action of the belladonna drugs.

Fig. 1. Mean changes in heart rate with atropine sulfate and atropine methylbromide without anesthesia. Twice as much atropine sulfate was required to produce acceleration of heart rate equal to that after atropine methylbromide. Heart rate slowed significantly (P < 0.05) after low doses of either drug, and a significantly greater slowing occurred for atropine sulfate than for atropine methylbromide (P < 0.05).

Electrical stimulation both drugs once again caused prolongation of P–R interval (fig. 8). P–R intervals had returned to normal once the atropine drugs had completely blocked the vagus. In figure 8, arrows 1 and 3 indicate the doses at which peak prolongation of P–R intervals occurred simultaneously with partial vagal blockade. Here again atropine methylbromide was more potent in producing prolongation of P–R intervals.

Discussion

Our findings confirm previous observations that atropine methylbromide is more potent than atropine sulfate.\(^\text{18}\)

Fig. 2. Mean changes in heart rate with atropine methylbromide with and without neostigmine premedication, during alpha-chloralose anesthesia. After neostigmine, twice the dose of atropine methylbromide was required to produce an acceleration of heart rate equal to that after atropine methylbromide alone.
Prolongation of the P–R interval can be considered a parasympathomimetic effect because, in the absence of drugs, prolongation concurrent with slowing of the heart rate is seen with vagal stimulation. For this reason we should anticipate a decrease in heart rate (rather than the observed increase) simultaneous with an increase in P–R interval with low doses of belladonna drugs. Since this did not occur it is possible that the P–R interval and the heart rate were influenced by different mechanisms. That prolongation of the P–R interval occurred simultaneously with a quickening (rather than slowing) of heart rate, i.e., two apparent opposing forces operated at the same time, may have found an expression in the occurrence of arrhythmias.

Premedication with neostigmine shifted the dose–response curves for heart rate significantly to the right, but had only an insignificant effect on P–R intervals. Neostigmine, an inhibitor of cholinesterase, is thought to act here primarily by making more acetylcholine available. Because neostigmine greatly influenced the action of the belladonna drugs on heart rate, but not so much on P–R interval, we might conclude that heart rate but not P–R interval was governed by the acetylcholinergic receptor system. Our data are not adequate to confirm this. It is, for instance, possible that the cholinergic system governing heart rate is more sensitive to neostigmine than that influencing the P–R interval and that larger doses (not tested), would have shown definitive effects of neostigmine on P–R interval.

We must reject our original hypothesis and conclude that atropine methylbromide, presumably without access to the central nervous system, was capable of exerting peripheral (rather than central) parasympathomimetic effects on the heart; it slowed the heart rate in the awake animals and prolonged P–R interval in both awake and anesthetized animals. Except for a difference in potency, atropine sulfate was indistinguishable from atropine methylbromide in these respects. We conclude that atropine sulfate also had a peripheral parasympathomimetic effect in the dog if we may call the prolonging effect on P–R interval (with electrical vagal stimulation, in vagotomized dogs) a parasympatho-mimetic action. Whether or not atropine sulfate exerted a central effect, in addition, cannot be determined from our data. The site of the peripheral effects, whether ganglionic or postganglionic, is not apparent from our experiments.

One difference between atropine sulfate and atropine methylbromide might be cited in tenuous support of a central vagal effect for atropine sulfate: atropine sulfate consistently produced slightly more slowing of heart rate in the awake animal than atropine methylbromide. Since atropine sulfate was no more effective on P–R interval or in accelerating heart rate to peak levels than atropine methylbromide, the greater effect of atropine sulfate in slowing heart rates may be secondary.
FIG. 4. Mean change in heart rate and P-R interval with alpha-chloralose anesthesia and atropine sulfate. Doses 1, 3, and 11 correspond to 0.0003, 0.003, and 0.03 mg/kg, respectively (see table 1). Peak prolongation of P-R interval occurred at dose 7 (0.01 mg/kg), during the acceleration phase of heart rate.

FIG. 5. Mean change in heart rate and P-R interval under alpha-chloralose anesthesia. Atropine methylbromide was given. Doses 1, 3, and 11 correspond to 0.00033, 0.0033, and 0.033 mg/kg (see table 1). Peak prolongation of P-R interval occurred at dose 5 (0.0066 mg/kg), during the acceleration phase of heart rate.
Fig. 6. Mean change in heart rate and P-R interval with atropine sulfate and no anesthesia. Doses 1, 3, and 11 correspond to 0.0003, 0.003, and 0.03 mg/kg (see table 1). Peak prolongation of P-R interval occurred at dose 8 (0.015 mg/kg), during the acceleration phase of heart rate.

Fig. 7. Mean change in heart rate and P-R interval with atropine methylbromide and no anesthesia. Doses 1, 3, and 11 correspond to 0.00033, 0.0033, and 0.033 mg/kg (see table 1). Peak prolongation of P-R interval occurred at dose 5 (0.0066 mg/kg) during the acceleration phase of heart rate. Significantly less (P < 0.05) atropine methylbromide (0.0066 mg/kg) was required to produce peak prolongation of P-R interval (compare Figs. 8 and 9).
to a central effect not shared by atropine methylbromide.

Summary

Changes in heart rate and P–R interval were studied in dogs after incremental doses of atropine sulfate and atropine methylbromide with and without premedication with neostigmine. In awake animals both atropine sulfate and atropine methylbromide in low doses produced slowing of heart rate. Chloralose anesthesia abolished this effect. In high doses, both drugs accelerated heart rate; simultaneously, P–R intervals were first prolonged, then shortened. Chloralose did not affect this action. The belladonna drugs prolonged P–R interval even in vagotomized animals, in whom the distal vagal stump was stimulated electrically. Results of the experiments suggest the existence of peripheral parasympathomimetic effects of atropine sulfate and atropine methylbromide in the dog.

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References


Drugs

NARCOTICS The effects of fentanyl and meperidine on minute volume, tidal volume and respiratory frequency at a controlled alveolar carbon dioxide tension of 46 mm. Hg in six normal men were determined at intervals during a four-hour period. Fentanyl was administered intramuscularly in two doses (0.1 mg. and 0.2 mg.) and meperidine in one dose (75 mg.) and both were compared with 2 ml. physiologic saline solution. The magnitudes of maximal depression by 0.1 mg. fentanyl and 75 mg. meperidine were similar. Equal analgesia was produced by 0.1 mg. fentanyl and 65 mg. meperidine. Therefore, the maximal ventilatory depression with fentanyl is slightly greater than that of meperidine. However, onset and peak depression of minute volume, as well as recovery toward control levels, occurred more rapidly with fentanyl. (Downes, J. J., Kemp, R. A., and Lambertsen, C. J.: The Magnitude and Duration of Respiratory Depression due to Fentanyl and Meperidine in Man, J. Pharmacol. Exp. Therap. 158: 416 (Dec.) 1967.)

BARBITURATE TOXICITY Acetylsalicylic acid (ASA) was administered orally to rats with pentobarbital or thiopental given intraperitoneally one hour later. Both sleeping time and mortality were significantly increased with the ASA—barbiturate sequence. A dose—response relationship was observed for both sleeping time and mortality after ASA—thiopental, but only for sleeping time after ASA—pentobarbital. Starvation prolonged thiopental sleeping time. The mechanism whereby ASA prolongs barbiturate sleeping time is unknown. Possibly ASA inhibits biotransformation of thiopental and pentobarbital. (Coldwell, B. B., and Peters, J. M.: Sleeping Time and Mortality of Rats Administered Acetylsalicylic Acid and Barbiturates, Canad. J. Physiol. 46: 47 (Jan.) 1968.)