Pharmacology Biochemistry & Behavior, Vol. 32, pp. 175–179. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Comparing the Cardiac Vagolytic Effects of Atropine and Methylatropine in Rhesus Macaques[1,2]

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Received 29 April 1988

JANSEN, H. T. AND J. A. DELLINGER. *Comparing the cardiac vagolytic effects of atropine and methylatropine in rhesus macaques.* PHARMACOL BIOCHEM BEHAV 32(1) 175–179, 1989.—Atropine and methylatropine (190 nmol/kg) were compared in rhesus monkeys (*Macaca mulatta*) for their ability to produce a cardiac vagal blockade using a noninvasive estimate of respiratory sinus arrhythmia (RSA). Twelve monkeys received both drugs via intravenous (IV) and intramuscular (IM) routes of administration and were monitored for 3 hr after treatment. Both drugs, regardless of the route of administration, reduced RSA amplitude. At this dose, methylatropine was more effective than atropine in its ability to reduce RSA amplitude, heart period (HP; beat-to-beat interval), and overall heart period variability (HPV). Estimated RSA amplitude and HPV returned to basal levels significantly earlier after IM atropine administration than after IV treatment. Methylatropine did not exhibit any route effects. In addition, the mean decrease in RSA amplitude and HPV for the IM route of atropine sulfate was significantly less than that for the IV route. Serum atropine concentrations correlated significantly with all variables after IM treatment but only with RSA and HPV after IV treatment. Methylatropine may therefore be more useful than atropine as a pharmacologic challenge drug for detecting organophosphorus (OP) exposure because of its longer duration of action, lack of route of administration differences, and less likelihood of crossing the blood-brain barrier. Further studies are needed to fully evaluate methylatropine's potential in the challenge method of OP detection.

Atropine sulfate Methylatropine Vagal tone monitoring Respiratory sinus arrhythmia

ATROPINE is widely used in the treatment of anticholinesterase toxicosis. We have recently shown that atropine may be used as a challenge drug in the detection of anticholinesterase inhibitors. In these studies a noninvasive estimate of respiratory sinus arrhythmia (RSA) was used to show an attenuated response to atropine after exposure to dichlorvos and fenthion in dogs (5,6) and pyridostigmine in monkeys (3). The doses of the cholinesterase inhibitors resulted in 40 to 50% erythrocyte acetylcholinesterase inhibition and produced no clinical signs of exposure, yet altered the vagolytic responses to atropine. The use of atropine as a challenge drug may, however, result in behavioral and cognitive disruptions related to its entry into the central nervous system (CNS) (2, 7, 8, 13, 16). We sought an antimuscarinic compound less likely to cross the blood-brain barrier yet be able to reliably produce vagolytic effects. Such a compound would be more suitable as a pharmacologic challenge drug in detecting the altered RSA response following exposure to cholinesterase inhibitors.

Numerous synthetic antimuscarinic compounds have been produced in recent years, many for clinical use. Several derivatives and analogs of atropine are used and include the scopine-containing scopolamine, methylatropine, and the mandelic acid-containing homatropine. Methylatropine was chosen for this study because of its structural similarity to atropine, its similar mode of action, and because of its expected lack of central effects due to the quaternary ammonium group.

Based on our previous work with rhesus monkeys and atropine (11), a noninvasive estimate of RSA amplitude was used to quantitatively compare the vagolytic effects of atropine and methylatropine at the heart. This method, developed by Porges (18), uses a standard electrocardiogram (ECG) signal and a Vagal Tone Monitor® (VTM; Delta

[1]This research was supported by the U.S. Air Force under a subcontract from the University of Utah, Utah Consortium for Research and Education, contract number 433615-83-D0603, Task 18.

[2]The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as official Department of the Air Force position, policy, or decision, unless so designated by other documentation.

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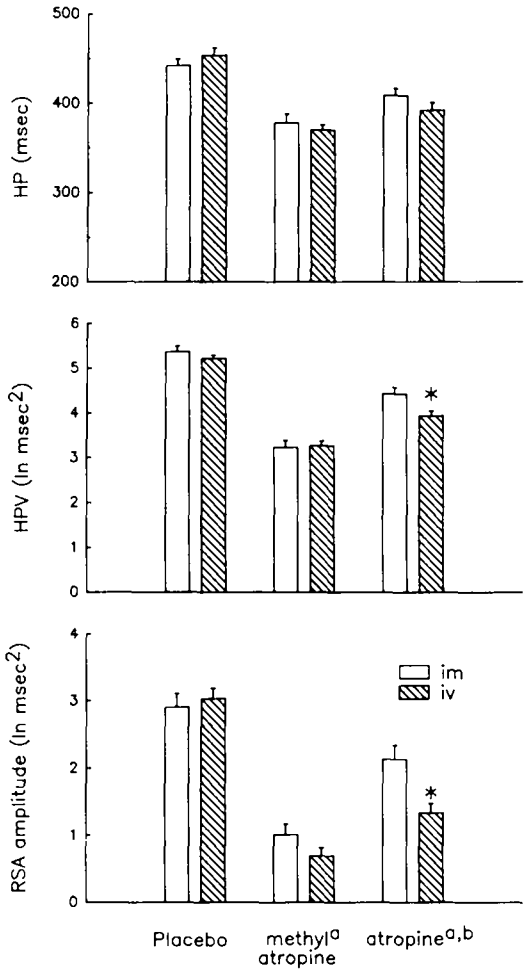

FIG. 1. Mean response by HP, HPV, and RSA amplitude in rhesus monkeys receiving IM and IV atropine and methylatropine (190 nmol/kg) or a saline placebo (±SEM). (a, significantly different from the placebo; a,b, significantly different from the placebo and methylatropine; *significantly different from IM; $p<0.05$ for all comparisons.)

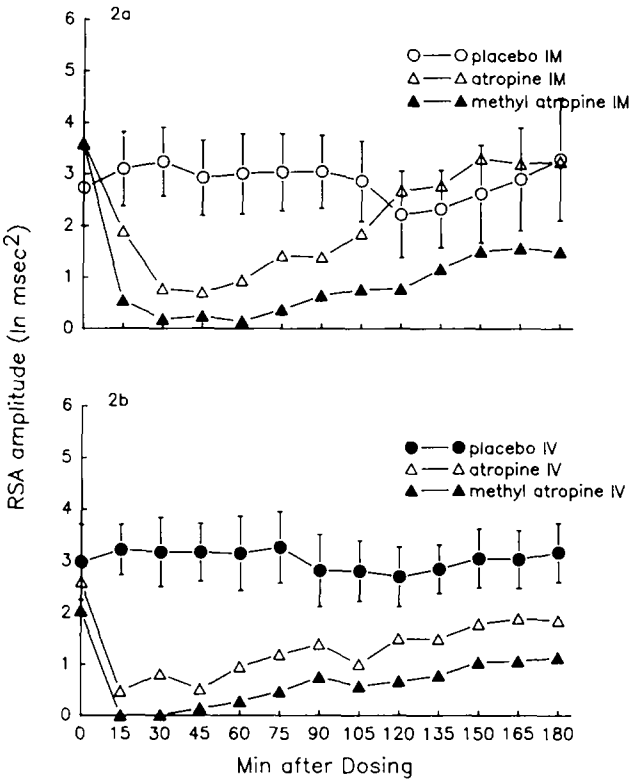

FIG. 2. (a–b) Mean responses over time for estimated RSA amplitude in 12 rhesus monkeys receiving IM (a) or IV (b) atropine or methylatropine (190 nmol/kg) or a saline placebo (±SEM). Times=0 represents the mean of 30 min of baseline data.

Biometrics, Bethesda, MD) to quantify the amplitude of HP variability occurring within a predetermined respiratory frequency. The RSA is thought to be a centrally mediated phenomenon (14, 15, 17) with afferent information being integrated at several CNS loci followed by efferent impulses sent, via the vagus nerve, to the heart. The RSA is manifested as an increase in heart rate during inspiration due to an active vagal inhibition and a decrease during expiration due to vagal excitation (9). Yongue *et al.* (22) have shown that the phenylephrine-induced hypertension in rats results in a reflexive increase in RSA. Our laboratory (7,11) has shown in man and monkeys that atropine abolishes the expression of RSA.

Heart rate has been shown to correlate highly with serum atropine concentrations by Gupta and Ellinwood (10) after IM administration but not by Adams *et al.* (1) who gave the drug IV. Recently, the pharmacokinetic parameters for IM and IV atropine have been described in man (19) and rhesus monkeys (4). Our previous studies have shown that similar doses of atropine are required to decrease RSA and HPV in monkeys when compared to man (11).

The first objective of this study was to determine whether the vagolytic effects of methylatropine are similar to those of atropine sulfate in rhesus monkeys, thereby making methylatropine a possible substitute for atropine as a pharmacologic challenge drug. Our second objective was to correlate HP, HPV, and the estimate of RSA amplitude with IM and IV serum atropine concentrations in rhesus monkeys and to compare the pharmacokinetic parameters with those described for the human as an indicator of the usefulness of the monkey model.

METHOD

Twelve captive born, young adult to adult male rhesus monkeys were used in this study. Animals were handled and used according to the "Guide for the Care and the Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council. Each monkey underwent 30 hr of restraint acclimation approximately one year prior to the present study. All of the monkeys were subsequently used in other cholinergic pharmacology studies. Four-week periods separated these experiments with an additional 4-hr acclimation period preceding each new experiment.

The use of Latin square designs in our earlier studies ensured that each monkey received all treatments. Signifi-

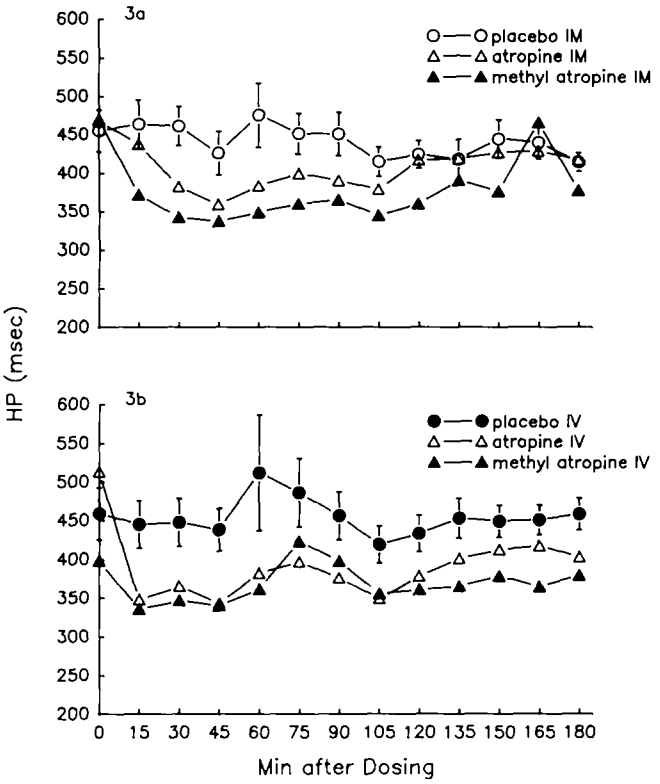

FIG. 3. (a–b) Mean responses over time for HP in 12 rhesus monkeys receiving IM (a) or IV (b) atropine, methylatropine (190 nmol/kg) or a saline placebo (±SEM). Time=0 represents the mean of 30 min of baseline data.

cant crossover effects were not observed in any of these experiments and the monkeys were therefore randomly reassigned to one of three groups (four animals per group) in a balanced incomplete block design for the present study. Equimolar (190 nmol of base) concentrations of atropine (sulfate; Med Tech Inc., Elwood, KS) and methylatropine (nitrate; Sigma Chemical Co., St. Louis, MO) or a saline placebo were administered IM or IV in a volume of 0.1 cc per kg of body weight. One-half hr of baseline data were collected prior to dosing followed by a 3 hr period of postdosing data collection.

A standard lead III electrocardiographic (ECG) configuration was used to collect the heart rate data which were confirmed on a strip chart recorder and sent to a dual channel oscilliscope for signal amplification and further analysis. A bellows and pressure transducer were used for respiratory rate determinations and also recorded.

The ECG and respiratory data were recorded on a four-channel cassette recorder after amplification and either stored (respiration) or processed in real-time using a VTM (ECG). The VTM digitizes the ECG data and performs a stepwise movement of a 21-point cubic polynomial through the HP data to calculate the RSA amplitude. The RSA amplitude is estimated as the heart period variability occurring within a respiratory frequency band of 0.12 to 0.40 Hz (human adult) or 0.30 to 1.3 Hz (human neonate) depending on the preset respiratory frequency range for each monkey. Overall HPV and RSA amplitude were converted to natural logarithms (ln) to normalize their distributions. Estimates of each variable (HP, HPV, and RSA amplitude) were computed every 30 sec and the data then summarized over 15 min for subsequent statistical comparisons.

Serum atropine concentrations were determined using the radioimmunoassay method of Wurzburger *et al.* (21). Blood samples were collected at 30, 90, and 180 min after dosing and the data hand-fitted for pharmacokinetic analysis. Comparisons between the areas under the concentration curves (AUC) for the IM and IV routes enabled an estimate of the overall drug bioavailability to be made.

A general linear model (GLM) procedure (SAS Institute Inc., Cary, NC) was used to perform a univariate analysis of variance (ANOVA) to test the main effects (animal—group, group, time, route, drug, and week) and the interactions between these effects. Differences between the main effects were determined using a Tukey's Studentized Range Test. All tests were performed at a 5% level of significance.

RESULTS

The overall responses to atropine and methylatropine when administered IM or IV are depicted in Fig. 1. No significant week (crossover) effects were observed. Both atropine and methylatropine depressed HP, HPV, and estimated RSA amplitude. Methylatropine reduced all three parameters to a greater extent than did atropine. Estimated RSA amplitude was deemed more sensitive than HPV or HP to the effects of both drugs since it was decreased to a greater extent when compared to its placebo value. Route of administration effects alone were not significant, yet route interacted significantly with drug for both HPV, $F(2,253)=20.15$, $p<0.0001$, and estimated RSA amplitude, $F(2,253)=7.59$, $p<0.0001$. Only atropine produced significant differences between IM and IV routes of administration ($p<0.05$).

The time course of the estimated RSA amplitude and HP responses to atropine and methylatropine are illustrated in Figs. 2a,b and 3a,b respectively; the shape of the HPV response curve is similar to that of estimated RSA amplitude and is therefore not shown. No changes in respiratory rates were observed during the experimental sessions. Drug and time effects were significant, $F(2,253)=196.85$, $p<0.0001$; $F(12,71)=10.74$, $p<0.0001$, respectively. Drug also interacted significantly with time, $F(24,253)=3.94$, $p<0.0001$. This difference can be most clearly seen for atropine during which the response by estimated RSA amplitude returned to basal levels after 105 min for the IM treatment vs. 150 min for the IV treatment. The HP responses were generally more variable, especially after IV drug or placebo administration, and resulted in substantial fluctuations throughout the 3-hr experimental sessions. These deviations may represent the effects of blood sampling. Such fluctuations were not manifested in estimated RSA amplitude or in HPV even though both parameters are estimated from HP data.

The pharmacokinetic parameters for atropine are given in Table 1. Methylatropine was not detectable by the RIA methods used. All parameters were similar for the two routes of administration with the IV results being generally more variable. The similarity between the AUC for the two routes of administration suggests that nearly 100% of the drug is bioavailable. Atropine produced its effects on all three parameters significantly earlier when given IV than when given IM ($p<0.05$). This was expected since plasma concentrations require approximately 1 hr to reach maximal levels after IM

TABLE 1

MEAN (±SD) CALCULATED PHARMACOKINETIC PARAMETERS FOR ATROPINE ADMINISTERED IM AND IV (190 nmol/kg)

Parameter	IM (N=6)	IV (N=6)
Ka (min $^{-1}$)	0.04 (0.012)	—
Kel (min $^{-1}$)	0.005 (0.002)	0.004 (0.003)
$t^1/_2$ abs (min)	19.8 (7.2)	—
$t^1/_2$ elim (min)	148.2 (57)	212.4 (100.2)
Vd (L/kg)	2.47 (1.01)	2.85 (1.38)
AUC (ng/ml/hr)	40.81 (4.26)	51.49 (17.8)

administration versus the nearly instantaneous maximum attained following IV administration. Atropine (IM) plasma concentrations correlated significantly with HP, HPV, and estimated RSA amplitude ($r=-.59, -.56, -.61$; $p<0.05$, respectively). Atropine (IV) plasma concentrations correlated only with HPV and estimate RSA amplitude ($r=-.76, -.55$; $p<0.05$, respectively).

DISCUSSION

Atropine and related alkaloids are generally regarded as specific muscarinic antagonists with their corresponding quaternary ammonium derivatives being more potent than the parent compound (12,20). The classical peripheral effect of antimuscarinic agents on the heart is to increase mean heart rate. Both atropine and methylatropine produced a tachycardia (Fig. 3a,b). In addition, we confirmed the relative potency of methylatropine versus atropine using all three parameters (HP, HPV, and estimated RSA amplitude). The parasympatholytic effects of atropine and methylatropine on the heart were determined with greater sensitivity using the amplitude of RSA than with HPV or HP. That is, the decrease seen after drug administration when compared to the placebo was greater for RSA than for HP or HPV. For example, a 71% decrease for RSA versus a 38% and 17% decrease for HPV and HP, respectively, after methylatropine was observed (Fig. 1).

The pharmacokinetics of atropine determined in this study (Table 1) are similar to those recently reported for the human and the rhesus monkey (4, 10, 19). Heart period, HPV, and RSA amplitude correlated significantly with serum atropine concentrations after IM administration. This route of administration produces a serum concentration curve that resembles the tissue concentration curve described by Adams *et al.* (1). The lack of correlation for HP after IV atropine administration may be due to compensatory effects at the heart (possibly sympathetic) during the rapid distribution of atropine from the plasma into the tissue compartment in order to maintain a constant mean heart rate. However, the correlation between estimated RSA amplitude, HPV, and plasma atropine concentrations indicates that little reflexive control exists to modulate the effects of a rapid muscarinic blockade at the heart. Such a blockade prevents the manifestation of RSA and HPV by effectively dissociating the vagus from the SA node.

In the monkeys used in this study, stressing manipulations such as venipuncture cause dramatic fluctuations in HP yet affect HPV (not shown) and RSA amplitude to a lesser degree. These results suggest that the latter may be able to respond independently of other nonvagal influences, such as sympathetic activity, which may affect mean heart rate only. The use of RSA amplitude as an indicator of anticholinergic drug efficacy and potency may, therefore, lend itself to a wide variety of clinical and experimental situations in which sufficient acclimation or training time is not possible.

Atropine treatment is widely recommended in the treatment of confirmed OP exposure and poisoning. An attenuated response to a challenge dose of methylatropine would necessitate treatment with atropine; however, in the absence of an attenuated methylatropine response the risk of behavioral alterations associated with atropine treatment alone may be avoided and awaits further study.

ACKNOWLEDGEMENTS

The authors are greatly indebted to D. Zaber, S. Birnbaum and S. Singh for their excellent technical assistance and to Dr. R. C. Smallridge at Walter Reed Army Institute of Research, Washington, DC, for performing the serum atropine RIA.

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