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Alterations in the Effects of Atropine on the Behavior of Pigeons Following Chronic Administration[1]

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SCHAAL, D W AND M N BRANCH *Alterations in the effects of atropine on the behavior of pigeons following chronic administration* PHARMACOL BIOCHEM BEHAV **28**(3) 375–380, 1987 —The acute effects of atropine (0 01–1 0 mg/kg) on pigeons' key-pecking maintained under a variable-interval (VI) 60-sec schedule of food reinforcement were determined Atropine decreased key-peck rates in a dose-dependent manner A rate-decreasing dose of physostigmine, an acetlycholinesterase inhibitor, was studied in combination with the range of atropine doses The rate reduction produced by physostigmine was attenuated by some doses of atropine An atropine dose which decreased key-peck rates was then administered to the pigeons every day after their experimental sessions (chronic post-session phase) During this regimen, the dose-effect curves for atropine and the combinations of atropine and physostigmine were redetermined Atropine was then given chronically prior to experimental sessions (chronic pre-session phase), and the dose-effect curves for atropine and the combinations of atropine and physostigmine were determined again The pigeons became tolerant to the rate-reducing effects of atropine following chronic post-session administration Physostigmine's effect alone was unchanged following chronic atropine administration for two pigeons, and was slightly greater for a third The rate reduction caused by physostigmine was attenuated across a wider range of atropine doses in the two pigeons for which the effect of physostigmine alone was unchanged. The atropine/physostigmine interaction curve for the third pigeon was shifted to the right following chronic post-session atropine administration No further changes in effects of either atropine alone or in combination with physostigmine were seen following chronic pre-session atropine administration Tolerance to atropine's rate-suppressing effects in this preparation, then, did not depend on the interaction of the pigeons while drugged with factors specific to the experimental situation, i e , it was not "contingent" or "behavioral" tolerance The tolerance exhibited some specificity, however, in that initially effective doses continued to antagonize the suppressive effects of physostigmine in two of three pigeons That is, although larger doses of atropine were required to suppress key-pecking, they were not required to antagonize physostigmine's effects

Acetylcholine Acetylcholinesterase Antagonism Anticholinergic Anticholinesterase
Antimuscarinic Atropine Key-peck Physostigmine Pigeons Tolerance Variable-interval

ATROPINE is a competitive antagonist of acetylcholine (ACh), it blocks synaptic transmission primarily at muscarinic receptor sites [25] Physostigmine is an acetycholinesterase (AChE) inhibitor, and thus prevents the enzymatic degradation of acetylcholine at all cholinergic receptor sites [22]. Their opposite receptor effects (antagonist vs. agonist-like) has prompted much research aimed at elucidating the specificity of their respective pharmacologic mechanisms of action. Some of this research has used behavioral measures as dependent variables. For example, Vaillant found that pigeons' key-pecking under a multiple fixed-interval (FI) fixed-ratio (FR) schedule that was completely suppressed by about 0.25 mg/kg physostigmine could be restored to control levels by 0.03 mg/kg atropine [23]. The same dose of physostigmine was found to suppress completely discriminated shuttle box avoidance in cats, an effect attenuated by 1 2 mg/kg atropine [12] Physostigmine (0 1 mg/kg) suppressed rates of lever-pressing by dogs maintained under an FR 1 schedule of intra-cranial brain stimulation across a range of current levels, an effect again attenuated by 0.2 mg/kg atropine [20] Physostigmine also has been found to alleviate the deleterious effects of scopolamine (a drug with pharmacologic actions similar to atropine) on humans' remembering of new word lists [13,17]. It is well established, then, that atropine and other antimuscarinics can antagonize behavioral effects of physostigmine across a range of procedures and species

Although the effects of atropine and physostigmine have received some attention by behavioral pharmacologists, atropine's effects following chronic administration have received very little, although those of scopolamine have received some (e.g., [2, 9, 10, 18]). Of particular interest is the

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question of whether tolerance to atropine's behavioral effects will develop during chronic administration, and, if so, will that tolerance depend on subjects encountering some of the environmental variables that control the measured behavior while drugged, i e., will the tolerance be "behavioral" or "contingent" tolerance [6,11].

The procedure employed here was modeled after the one described by Branch [4] He examined tolerance to pentobarbital in single subjects by determining dose-effect curves acutely, then again following chronic post-session drug treatment, and finally after a second chronic regimen of pre-session drugging. In this procedure, if tolerance to the behavioral effects of the drug is not apparent after the post-session drug treatment but develops as a result of pre-session chronic drug administrations, then the tolerance is said to be behavioral A similar procedure was employed here to allow within-subject comparisons with atropine

A second purpose of the present study was to determine the extent of atropine's ability to antagonize behavioral effects of physostigmine, and to see if this antagonism changes following chronic atropine administration

METHOD

Subjects

Three male, White Carneaux pigeons, approximately 8 years old, served as subjects. The pigeons had participated in an experiment on briefly-signaled delay of reinforcement using the same baseline reinforcement schedule as the one used in the present experiment, but had never been given drugs. They were deprived of food to approximately 80% of their free-feeding body weights, and between sessions were housed individually in a temperature-controlled colony with a 16-hr light, 8-hr dark cycle They had free access to water and digestive grit when not in an experimental session

Apparatus

Experimental sessions were conducted seven days a week in a custom-built conditioning chamber for pigeons, 30 cm wide by 31 cm long by 31 cm deep. The front wall was a brushed-aluminum three-key operant panel equipped with a standard mixed grain feeder below the middle key and two houselights mounted in either upper corner. The middle key, the only one used in this experiment, required a force of 0.14 N to operate a feedback relay and be recorded as a response White masking noise and the sound of a ventilating fan were continuously present. A minicomputer, located in an adjacent room, programmed the contingencies and collected the data A Gerbrands cumulative recorder provided continuous records of key-pecking.

Behavioral Procedure

Since the pigeons' key-pecking had been shaped and maintained for a previous experiment, no prior training was necessary. Pecks to a translucent response key lighted from behind with a green light produced 4 sec access to mixed grain according to a variable-interval (VI) 60-sec schedule constructed from the constant-probability distribution of Catania and Reynolds [7]. Sessions began 10-min after the pigeon was placed in the chamber, during which time the chamber was dark and the key was not operative The houselights and key-light were then illuminated, and key-pecking could commence. Houselights and key-light were extinguished and a feeder light was illuminated during grain presentation Sessions ended after 30 reinforcers, or after 45 min, whichever came first.

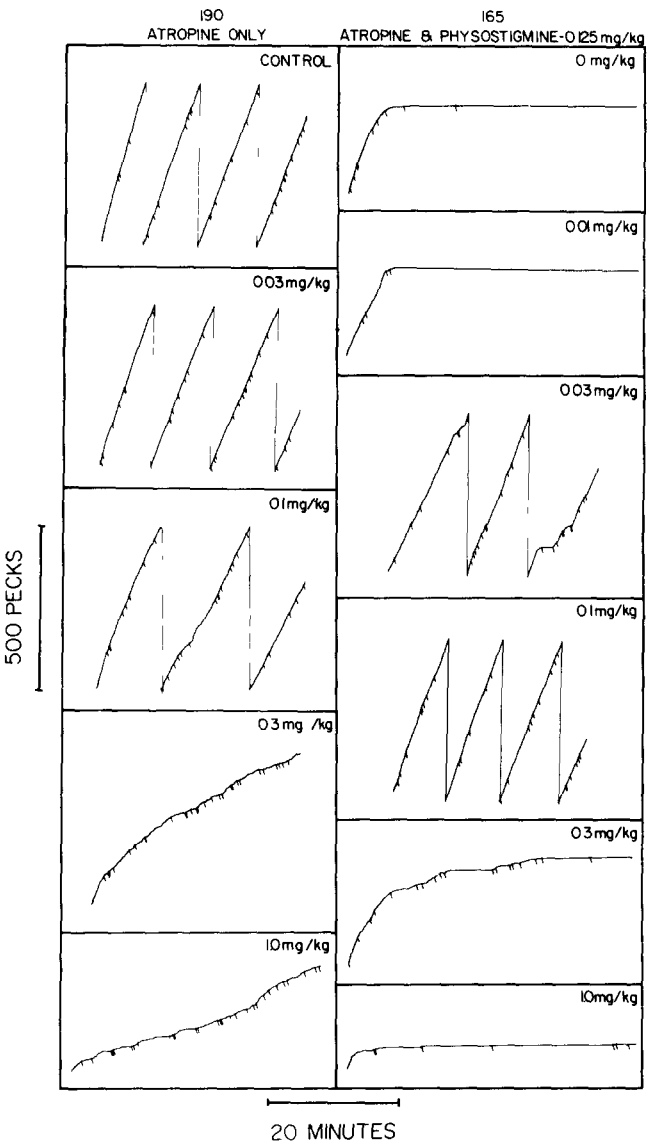

FIG 1 Cumulative response records indicative of the performance of Pigeon 190 after administration of a range of atropine doses alone (left column) and Pigeon 165 after administration of 0 125 mg/kg physostigmine combined with the vehicle (sterile water, upper right frame, 0 mg/kg) and a range of atropine doses (right column) Records are taken from the acute administration phase Performance for a session in which no injection was given is shown by the record marked "CONTROL" (upper left frame) Pen deflections indicate mixed grain presentations

Drug Procedure

Atropine sulfate and physostigmine salicylate (obtained from the U.S Army Research and Development Command) were dissolved in sterile distilled water and injected into the breast muscle in a volume of 1 0 ml/kg body weight Dosages are expressed in terms of the salt

When response rates had become stable, effects of two ascending series of atropine doses (0.03–1 0 mg/kg for Pi-

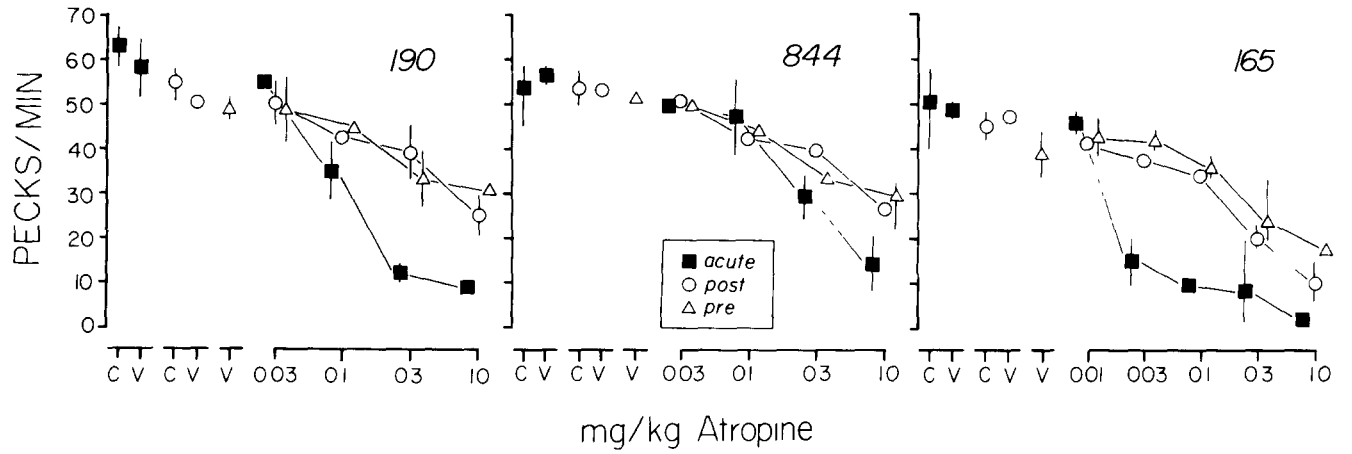

FIG 2 Effects of a range of doses of atropine sulfate on the key-peck rates of all three pigeons Filled squares depict the acute dose effects on key-peck rates, open circles depict dose effects after chronic post-session atropine administration, and open triangles depict dose effects after chronic pre-session atropine administration No-drug control rates, taken from sessions immediately prior to drug and vehicle administration sessions, are plotted over the letter C, and rates from sessions in which the vehicle (sterile water) alone was injected are plotted over the letter V Vertical lines on points indicate ranges, range bars are omitted where the point covers the entire range

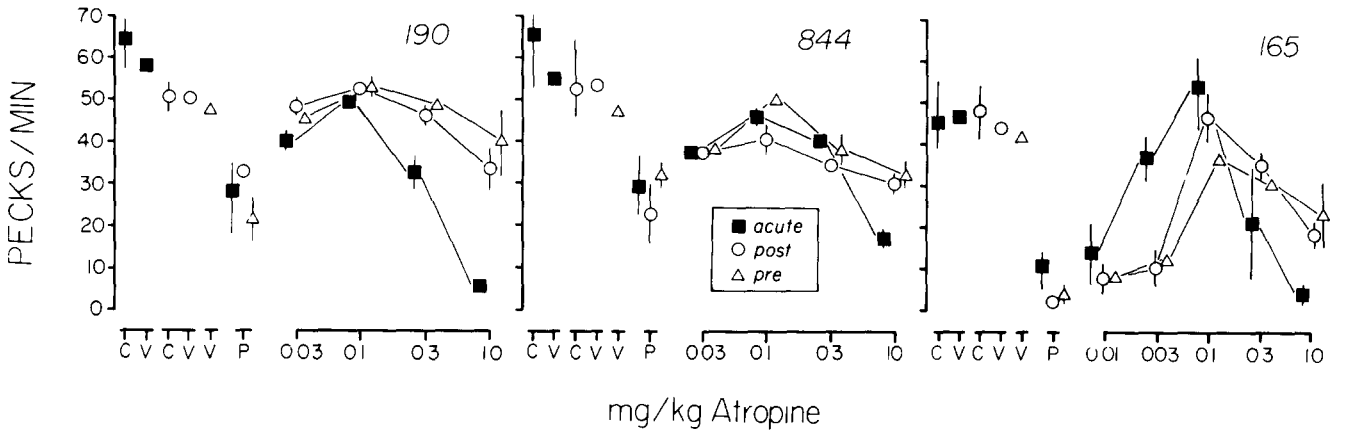

FIG 3 Effects of the combination of a single dose of physostigmine salicylate and a range of doses of atropine sulfate on key-peck rates of all three pigeons Physostigmine doses were 0 125 mg/kg for Pigeons 165 and 190, and 0 08 mg/kg for Pigeon 844 Filled squares indicate acute dose effects on key-peck rates, open circles depict dose effects after chronic post-session atropine administration, and open triangles depict dose effects after chronic pre-session atropine administration Effects of physostigmine alone are depicted for each condition above the letter P No-drug control rates, taken from sessions immediately prior to drug and vehicle administration sessions, are plotted above the letter C, and rates from sessions in which the vehicle (sterile water) alone was given are plotted above the letter V Vertical lines on points indicate ranges, range bars are omitted where the point covers the entire range

geons 190 and 844, 0.01–1.0 mg/kg for Pigeon 165, the lowest dose served to anchor the dose-effect curve at a dose that had no effect on response rates) and its vehicle (sterile water) were determined. Third determinations were performed as needed as extra checks on reliability. Doses were administered in ascending series to facilitate the observation of possible systematic changes in dose effects across repeated administrations. Injections were spaced by at least 3 days Following determination of the acute dose-effect curve, doses of physostigmine were administered until one that reduced key-peck rates below 50% of baseline but above zero was found for each pigeon (0.08 mg/kg for Pigeon 844, 0.125 mg/kg for Pigeons 190 and 165), this dose was the only one used in the rest of the experiment. Physostigmine was administered in combination (two injections, one in each breast muscle) with the vehicle and with each of the atropine doses twice, again in ascending order, to produce an interaction curve Again, doses were spaced by at least 3 days After these acute determinations a dose of atropine that reduced key-peck rates appreciably (1 0 mg/kg for Pigeons 190 and 844, 0.3 mg/kg for Pigeon 165) was administered every day about 30 min after experimental sessions (post-session chronic phase). Sham injections (insert needle but inject no fluid) preceded each session during this phase. After the first 20 sessions (days) of post-session chronic drug administration, the vehicle and two ascending series of atropine doses were administered pre-session (as "probes") in the same manner as acute administrations (that is, spaced at least three days apart). Intervening sessions continued to be followed by atropine administration Sham injections were

given about 30 min after each probe session. Physostigmine's effects alone and in combination with atropine were determined again during this phase in the same manner as above The chronic atropine dose was then administered prior to experimental sessions (pre-session chronic phase). After the first 20 sessions of this regimen the vehicle and the other atropine doses were substituted for the chronic atropine dose in two ascending series as before, with intervening sessions still preceded by administration of the chronic atropine dose. Physostigmine and atropine combinations were then substituted for the chronic atropine dose (in the same manner) to determine the final interaction curve

RESULTS

Acute effects of a range of atropine doses (for Pigeon 190, left column) and the combination of a single dose of physostigmine with a range of atropine doses (for Pigeon 165, right column) are illustrated in the cumulative response records in Fig. 1. Records are representative of the performance of all three birds Under non-drug conditions, performance typical of that usually observed under VI schedules was observed Key-pecking occurred at a steady, nearly constant rate The records show the progressive decrease in key-peck rates and disruption of steady, VI-like response patterns with increasing doses of atropine alone (left column) Physostigmine (0 125 mg/kg, right column, top frame) produced long periods with no pecks when administered alone Increasing doses of atropine attenuated physostigmine's rate-reduction maximally at 0 1 mg/kg, larger doses were less effective or combined to produce more complete suppression of rates

Figure 2 shows dose-effect curves for each subject over a range of atropine doses administered alone under acute conditions (filled squares), and during the post-session chronic (open circles) and pre-session chronic (open triangles) phases Under acute administration, atropine decreased rate as its dose was increased, with 1 0 mg/kg reducing rates to between zero and 20 pecks/min among subjects During the post-session chronic phase dose-effect curves were shifted to the right, indicating that the pigeons had become tolerant to the drug's rate-reducing effect. During the pre-session chronic phase the dose-effect curves were essentially unchanged from the post-session chronic phase.

Effects of the combination of a single dose of physostigmine with a range of atropine doses under acute conditions (filled squares), and during the post-session chronic (open circles), and pre-session chronic (open triangles) phases are presented in Fig 3 Across each condition the rate-reducing effect of physostigmine in combination with the sterile water vehicle (plotted for each condition above the letter P) was unchanged for Pigeons 190 and 844, but was greater for Pigeon 165 after each chronic phase relative to physostigmine's initial effect Acutely, 0 1 mg/kg atropine restored key-peck rates suppressed by physostigmine to baseline levels for each subject Following post-session chronic atropine administration the interaction curves were shifted to the right, either making atropine an effective antagonist across a wider range of doses (e.g., Pigeons 190 and 844) or making larger doses effective antagonists (e.g , Pigeon 165)

DISCUSSION

Atropine administered acutely produced dose-dependent decreases in rates of key-pecking for three pigeons responding under a VI 60-sec schedule of food reinforcement, and at small and moderate doses antagonized the rate-reducing effect of a single dose of physostigmine. Results obtained during acute administrations of atropine alone concur with the findings of other researchers who demonstrated dose-dependent decreases in response rates maintained under VI schedules of food or water reinforcement with rats [3,15] and squirrel monkeys [8], and under fixed-ratio (FR) schedules of food or water reinforcement in rats [3,26] and pigeons [24] In contrast, some doses of atropine have produced increases in rates of behavior maintained under fixed-interval (FI) schedules of food or water reinforcement in rats [3], and pigeons [24], under FI schedules of either food reinforcement or termination of a stimulus associated with impending shock in squirrel monkeys [16], and with behavior maintained under differential reinforcement of low rates (DRL) schedules of food or water reinforcement in rats [5,26] Results of acute administration of atropine suggest its effects are somewhat dependent on the control rate of responding [26]

Chronic post-session atropine administration resulted in tolerance to atropine's rate-reducing effects, and the tolerance was not augmented by subsequent chronic pre-session drug treatment Tolerance to atropine under these conditions, therefore, was not contingent upon interaction of the pigeons while drugged with variables specific to the experimental situation, i.e , it was not "behavioral" or "contingent" tolerance. Whether pre-session chronic administration would have resulted in a greater degree of tolerance had it preceded post-session chronic administration cannot be known given the present data Further experiments in which the order of chronic administration (pre-session vs post-session) is counterbalanced could reveal quantitative differences in the effects of these chronic regimens not revealed in the current study However, the present results concur with those of Charney and Reynolds [9,10], who found that tolerance developed to the rate-reducing and pattern disrupting effects of scopolamine, another anticholinergic drug, on the fixed-interval performance of two groups of rats maintained by water reinforcement, and that tolerance developed to a similar degree whether the drug was given prior to or after experimental sessions

Although Charney and Reynolds' results and those of the current study suggest that tolerance to the effects of these anticholinergic drugs develops independently of the influence of behavioral factors specific to the test situations, others have demonstrated rapid development of tolerance to scopolamine that was either not observed in subjects that were not allowed to interact with the experimental conditions while drugged [2,18] or was delayed [2] These findings do not preclude the possibility that extended exposure to the drug may result in tolerance without a contribution from drug-behavior interactions (as observed in the present study); they do show that under certain conditions a rapid tolerance to behavioral effects of scopolamine can develop, and that more than mere exposure to the drug is required to observe it (see [1] for a discussion of these divergent effects)

Physostigmine's effect in combination with sterile water was a reduction in key-peck rates that was unchanged after chronic atropine administration for Pigeons 190 and 844, but was slightly greater after chronic administration for Pigeon 165 Changes in the interaction curves following the chronic atropine regimens are more difficult to characterize than the changes in the dose-effect curves for atropine alone. For all three pigeons the rate-reduction caused by physostigmine in combination with 1 0 mg/kg atropine was considerably less

after chronic administration than it was when this combination was given acutely This is probably due to the fact that this dose of atropine given alone had much less of a rate-reducing effect following chronic drug treatment, so that antagonism of effects of physostigmine could be observed without being obscured by the severe rate-reducing effect of that atropine dose alone, i.e , the antagonism was "unmasked" (see [24]) For two subjects, 190 and 844, small doses of atropine continued to act as effective antagonists For Pigeon 165, by contrast, 0 03 mg/kg of atropine was no longer effective as an antagonist after chronic treatment, suggesting that tolerance to this action had developed. This interpretation, however, may be compromised by the fact that for this pigeon the effects of physostigmine were slightly greater following chronic treatment with atropine (though the difference in the reduction of response rates by physostigmine following chronic atropine administration may be too small to account for the changes in the interaction curves) The interaction curves, while complex in their pharmacological implications, nevertheless can be said to support the conclusion supported by the atropine-alone dose-effect curves. That is, pigeons became tolerant to the rate-reducing effects of atropine following chronic post-session drug treatment, and tolerance was essentially unchanged following chronic pre-session drug treatment. Atropine, however, still continued to act as an effective antagonist of physostigmine, indicating that little if any tolerance to this action of the drug had developed Therefore, even though the tolerance observed was contingent on behavioral tolerance according to accepted criteria, it still exhibited some specificity, tolerance developed to atropine's major behavioral effect but not (or at least, less so) to its effectiveness as an antagonist.

Although it appears that tolerance to atropine's effects did not depend on interaction with factors specific to the experimental situation while drugged, interpretation of the tolerance in terms of changes in cholinergic receptors becomes difficult when one considers that the effect of physostigmine alone did not change in two subjects (and changed little in the third) even though each subject became tolerant to atropine alone Although the current data cannot be used to draw conclusions regarding possible pharmacological mechanisms of the tolerance observed, several speculative interpretations exist. The degree of up-regulation of muscarinic receptors observed in the hippocampus, striatum and cerebral cortex of Sprague-Dawley rats [21], and in the same brain structures of guinea pigs [27] after chronic atropine administration is somewhat small, and has resulted from daily doses 20 times the chronic dose used in the present study If a similar increase in muscarinic receptors was produced by chronic atropine administration in the pigeons of the present study (a fact that could account for the tolerance observed), one might expect to observe greater sensitization to the behavioral effects of physostigmine in these animals than was observed However, chronic atropine administration also has been shown to result in muscarinic up-regulation in the periphery, Hedlund *et al* [14] produced a more than 100% increase in muscarinic receptor number in the salivary glands of Sprague-Dawley rats with the same daily dose used in the Yamada *et al* [27] study (which resulted in a 23% increase in striatal muscarinic receptors of guinea pigs). If atropine's effects on peripheral structures contribute to its rate-reducing action, and physiological tolerance to atropine develops more readily and to a greater extent in the periphery than centrally, the tolerance observed in the present study could have been a function of muscarinic up-regulation in the periphery Physostigmine's rate-reducing effect seems to involve central as well as peripheral mechanisms since atropine is less potent as an antagonist of the similar rate-reducing effect of neostigmine, a quaternary amine of physostigmine which crosses the blood-brain barrier with difficulty [23], and since methylatropine, atropine's quaternary amine, is less potent as an antagonist of this effect of physostigmine [24] Changes in muscarinic receptor number in the periphery would not be expected to change physostigmine's central effect.

Physostigmine prevents ACh degradation at nicotinic receptor sites as well as muscarinic sites, and thus nicotinic action may be involved in the physostigmine-induced rate-reduction observed One would not expect an increase in muscarinic receptor number to increase this suppressant effect if it is predominantly due to nicotinic effects Such an interpretation, however, does not account for the antagonism by atropine of physostigmine's behavioral effects in this and other studies (e g , [12, 19, 20, 23, 24]). Furthermore, Vaillant [24] showed that anti-nicotinic drugs mecamylamine and dihydro-β-erythroidine failed to antagonize physostigmine's response rate-reducing effect. This suggests that physostigmine's rate-reducing effect may be more highly correlated with its effects on muscarinic receptors than with its effects on nicotinic receptors

In summary, although tolerance to atropine's rate-reducing effect was produced with chronic administration, and this tolerance could not be called "contingent" or "behavioral," explanations in terms of muscarinic receptor changes are complicated by the lack of reliable changes in physostigmine's effect. Tolerance in the present study's subjects may have been correlated with up-regulation of peripheral muscarinic receptors, a change that would be less likely to result in a change in physostigmine's primarily central effect. While interpretations in terms of muscarinic receptors seem plausible, it is possible that chronic atropine administration could produce changes in the interaction of cholinergic with other neurochemical systems. Interestingly, Takeyasu *et al.* [21] found that atropine-tolerant rats were more sensitive to the activity-increasing effect of apomorphine, a dopaminergic agonist, than untreated controls. Descriptions of atropine tolerance in terms of such complex neurochemical interactions, as well as the explanations already suggested, await empirical investigation

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