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Time-Dependent Changes in the Effects of Cholinesterase Inhibitors on Shuttle-Box Avoidance

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GONZALEZ, L. P. AND H. L. ALTSHULER. *Time-dependent changes in the effects of cholinesterase inhibitors on shuttle-box avoidance.* PHARMAC. BIOCHEM. BEHAV. 12(6) 847–850, 1980.—Physostigmine and neostigmine were compared for their effects on shuttle-box avoidance acquisition and retention. Physostigmine impaired acquisition at doses lower than neostigmine. Avoidance performance 1, 7, or 14 days after acquisition was impaired by the administration of 0.4 mg/kg physostigmine or an equimolar dose of neostigmine. The effects of lower doses of physostigmine, but not of neostigmine, were dependent upon the time of original training relative to drug administration and retesting. The results suggest that the peripheral effects of higher doses of cholinesterase inhibitors impair avoidance performance. The effects of lower doses of physostigmine on acquisition and the time-dependent effects on subsequent performance are probably due to the central actions of this drug.

Physostigmine Neostigmine Shuttle-box avoidance Cholinesterase inhibition

DEUTSCH [3,4] has proposed that training initiates time-dependent alterations in cholinergic synaptic mechanisms which are reflected in changes in performance [12]. The temporal characteristics of altered cholinergic activity have been examined through the administration of anticholinergic and anti-cholinesterase agents at various intervals after learning.

Evidence from several studies has suggested a relationship between time-dependent changes in central cholinergic functioning and information retrieval in the central nervous system. Pharmacologically-induced alterations in the activity of cholinergic neurons is reported to impair, improve, or have no effect upon performance depending upon the time of drug administration relative to task acquisition. These results have been observed for performance of discriminated escape tasks [5, 6, 9, 16, 17, 19], an operant escape task [1], appetitively-reinforced discrimination [21], and a passive avoidance task [10,11].

While the majority of studies of the effects of cholinergic drugs on behavior support the general conclusion of Deutsch [3], some investigations have not observed a relationship between the time of drug administration after training and performance [7,8]. Cox [2] reported time-dependent changes in the performance of saline-treated animals which paralleled the changes in performance of animals treated with the anticholinesterase physostigmine. In addition, the involvement of peripheral cholinergic mechanisms has been suggested [18].

The present study was conducted to extend the reported time-dependent effects of the anticholinesterase physostigmine to the acquisition and performance of an active avoidance task, to determine the dose-response characteristics of these effects, and to examine the involvement of peripheral cholinergic mechanisms.

METHOD

Subjects

Four hundred twenty male, 250–325 g Sprague-Dawley rats (Simonsen Laboratories) were used in this study. Animals were housed in individual stainless steel cages with free access to food and water for the duration of the experiment.

Apparatus

The training apparatus used in this experiment was a two-way shuttle-box consisting of a Plexiglas chamber (35×35×46 cm), with an aluminum grid floor enclosed within a sound attenuating chamber. The grid was balanced at the center of the chamber so that movement of a subject from

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one side of the chamber to the other caused the grid to tilt, thus providing a means of monitoring chamber crossings. The grid floor was connected to a scrambled shock source (0.8 mA, AC). The shuttle-box chamber was also equipped with a 2900 Hz tone generator (Sonalert). BRS/Foringer relay equipment was used to program the presentation of trials and to record response information.

Procedure

Subjects were placed in the shuttle-box for training. Following a 10 min adaptation period, a 5 sec, 2900 Hz tone was presented. If the animal responded during this period by crossing to the other side of the chamber, the tone was extinguished and the subsequent onset of foot shock was avoided. If a response was not made during the first five seconds of the tone, the tone remained on and 0.8 mA of AC shock were delivered to the grid floor of the chamber. These stimuli remained on until a response was made, at which time the tone and shock were both extinguished. Thirty seconds after the termination of a trial, a new trial was begun. Thus, the minimum shock-to-shock interval was thirty-five seconds. Trials continued until a subject avoided shock successfully on 10 successive trials or until 80 trials had been presented.

The subjects were divided into groups of 15 subjects each. Seven of these groups received drug pretreatment 30 min before initial training. The remainder were trained nondrugged but were treated prior to retesting several days after training. Drug pretreatment consisted of IP injections of one of the following: saline (1 cc/kg), physostigmine salicylate (0.4 mg/kg, 0.1 mg/kg, or 0.04 mg/kg), or neostigmine methylsulfate (0.3 mg/kg, 0.08 mg/kg, or 0.03 mg/kg). The doses of neostigmine methylsulfate are equimolar to those of physostigmine salicylate.

Animals trained with no drug pretreatment were tested for retention either 1, 7, or 14 days after original training. These retention intervals were selected so as to allow a comparison of the obtained results with those of Deutsch [3]. The drugs listed above were administered to different groups 30 min before retesting at each of the retention intervals. Retention testing consisted of retraining subjects to the same criterion (10 successive avoidances or 80 trials) used during training.

The number of trials required to reach criterion performance during acquisition and retraining were recorded for subsequent data analysis. Data were analyzed by means of an analysis of variance. Significant group differences were further analyzed with Duncan's Multiple Range tests.

RESULTS

The mean number of acquisition trials for subjects pretreated with drug 30 min prior to avoidance training are presented in Fig. 1. Physostigmine salicylate significantly ($p<0.001$) impaired acquisition at all three doses. Animals receiving 0.4 mg/kg physostigmine were unable to learn the avoidance response within the maximum number of trials allowed (80 trials).

Neostigmine methylsulfate impaired acquisition in a dose-related fashion. The administration of 0.03 mg/kg neostigmine had no significant effect upon the number of trails to criterion as compared to saline-injected animals. Doses of 0.08 mg/kg and 0.3 mg/kg both impaired acquisition with the higher dose resulting in significantly greater impairment ($p<0.001$).

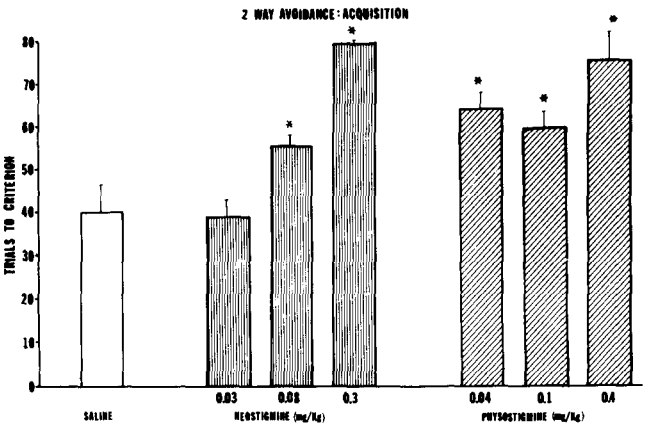

FIG. 1. Mean active-avoidance acquisition trials for subjects pretreated with saline, neostigmine, or physostigmine. Solid bars indicate standard error of the mean. *=significantly different from saline control ($p<0.05$).

Rats trained without prior drug treatment required significantly fewer retraining trials to reach the criterion of 10 successive avoidances when training was preceded by a saline injection (1 cc/kg) 30 min prior to retraining. Retraining trials to criterion were, on the average, 30% fewer than the number of trials required for original training. This difference in original training versus retraining trials to criterion was significant for animals retrained 1, 7, or 14 days after original training. Saline groups retrained 1, 7, or 14 days after original training did not differ significantly from one another in retention, as measured by retraining trials to criterion. The mean retraining to criterion for all the groups are presented in Fig. 2.

Neostigmine methylsulfate was found to cause the same dose-related impairment in retraining that was observed with treatment prior to original training. Animals receiving 0.03 mg/kg neostigmine 30 min prior to retraining did not differ significantly from saline-injected animals in retraining trials to criterion at any of the retention intervals (1, 7, or 14 days). Groups receiving either 0.08 mg/kg or 0.3 mg/kg neostigmine showed significant impairment during retraining, the higher dose producing significantly greater impairment.

Physostigmine salicylate injected 30 min prior to retraining produced significant impairment at the dose of 0.4 mg/kg. This impairment occurred in animals retrained 1, 7, or 14 days after original acquisition, and was comparable to that produced by the equimolar dose of neostigmine, 0.3 mg/kg. This finding suggests that the effects of this dose of physostigmine on retention, as on acquisition, can be attributed to the peripheral actions of physostigmine. Animals receiving 0.1 mg/kg physostigmine prior to retraining were not significantly different from saline animals if they were retrained one day after acquisition, but this dose of physostigmine impaired retraining if given 30 min prior to a retention test 7 or 14 days after acquisition.

The lowest dose of physostigmine used in this study, 0.04 mg/kg, facilitated relearning one day after acquisition, so that these animals required significantly fewer retraining trials to reach the response criterion than did saline animals. The effect of this same dose was significant impairment at retraining 7 or 14 days after acquisition.

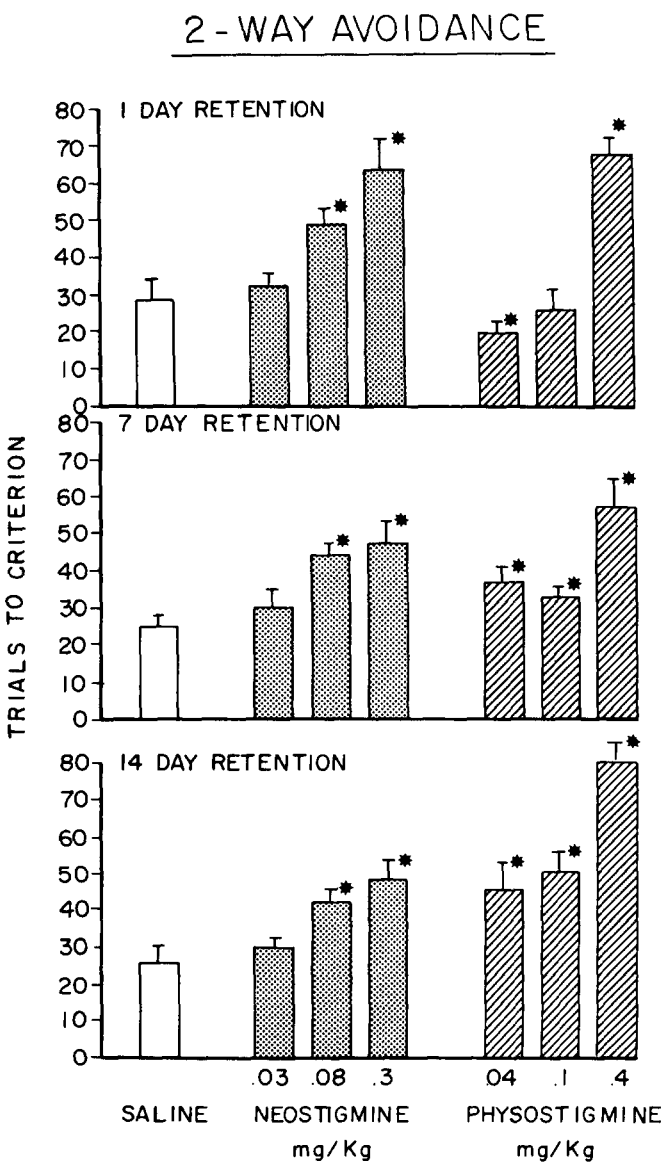

FIG. 2. Mean retraining trials to criterion one, seven, or fourteen days after original training for subjects pretreated with saline, neostigmine, or physostigmine. Solid bars indicate the standard error of the mean. *=significantly different from saline control ($p<0.05$).

DISCUSSION

Comparison of the behavioral effects of physostigmine with equimolar doses of neostigmine provides a means of distinguishing between the peripheral and central effects of cholinesterase inhibition. These drugs produce equivalent inhibition of AChE activity at equimolar doses [14], but the low permeability of neostigmine to the blood-brain barrier results in little central effect of this drug as compared to physostigmine [13,15]. The effects of systemic physostigmine could be due to central or peripheral AChE inhibition, but the effects of neostigmine result primarily from peripheral inhibition.

The two highest doses of physostigmine used in this study, 0.1 mg/kg and 0.4 mg/kg, impaired acquisition to an extent comparable to that of equimolar doses of neostigmine. This suggests that the impairment seen at the higher doses of physostigmine could be the result of their action at peripheral cholinergic sites. These results are similar to the findings of Rosecrans and Domino [15]. These investigators have reported impairment in the acquisition of a pole-jump avoidance task with physostigmine (0.1 and 0.15 mg/kg) as well as with neostigmine (0.12 mg/kg). Comparison of the antagonism of this impairment by central and peripheral cholinergic blockers led to the conclusion that at those doses physostigmine impaired avoidance acquisition primarily through a central action although peripheral effects also played a role in the alteration of behavior.

In the present study the lowest dose of physostigmine, 0.04 mg/kg, also impaired acquisition, while an equimolar dose of neostigmine did not. The impairment seen with this dose of physostigmine is thus probably due to a central component of physostigmine action. These results suggest that high levels of peripheral AChE inhibition impair active avoidance acquisition, but that similar impairment can be produced with much lower levels of central AChE inhibition.

Differential effects of peripheral versus central cholinesterase inhibition were also observed during tests of retention. Physostigmine was found to have a dose- and time-dependent effect on retention of active avoidance. Subjects receiving 0.4 mg/kg physostigmine at retention tests 1, 7, or 14 days after initial training showed significantly impaired performance. Since an equimolar dose of neostigmine produced the same impairment, this effect of 0.4 mg/kg physostigmine can be attributed to peripheral cholinesterase inhibition. At a 24 hr retention test, 0.04 mg/kg physostigmine facilitated performance; this dose impaired the performance of animals tested 7 or 14 days after training. An equimolar dose of neostigmine had no significant effect on performance at these retention intervals. Physostigmine at a dose of 0.1 mg/kg had no effect on avoidance at the 24 hr retention test, but impaired performance 7 or 14 days after original training; neostigmine at an equimolar dose impaired performance at each retention interval. The time-dependent effects of 0.1 mg/kg and 0.04 mg/kg physostigmine were not observed with neostigmine.

Results very similar to these have been reported for the effects of diisopropyl fluorophosphate (DFP) and physostigmine on retention of a discriminated escape task [5,9]. In these studies animals showed good performance when drug administration and retesting were 1 to 3 days after acquisition, but cholinesterase inhibition impaired performance 5 to 14 days after acquisition. These results were interpreted [3] to indicate that the process of learning initiates certain time-dependent changes in the sensitivity of cholinergic synapses. Such sensitivity is hypothesized to be initially low, increasing to a maximum at about two weeks after training, and then decreasing during the next few weeks. Potentiation of cholinergic functioning, as with physostigmine or DFP, aids performance when synaptic sensitivity is presumed to be low, but impairs retention when sensitivity is high.

In the present study 0.04 mg/kg physostigmine facilitated performance one day after initial training, when cholinergic sensitivity is assumed to be low [3]. This same dose impaired the performance of animals tested seven or 14 days after initial training, when the sensitivity of cholinergic synapses is hypothesized [3] to be high.

Since drug administration always immediately preceded retention testing in the present study, it is not possible to separate direct effects on retrieval mechanisms from alterations in other central performance mechanisms. In addition,

since animals were always initially trained in a non-drugged state, it is possible that the effects of drug administration on performance during retention testing include state-dependent effects [20]. Such an interpretation, dissociation due to a change in drug state, might explain the impairment seen with various doses of physostigmine; but, this would not provide an adequate explanation of the facilitation of performance seen with 0.04 mg/kg physostigmine or the lack of effect of 0.1 mg/kg physostigmine at the Day-One retention test, while both doses produced impairment at later retention tests.

Nevertheless, this study demonstrates that the acute effects of physostigmine on the performance of two-way shuttle-box avoidance are dependent upon the interval of time between initial training and retention testing, when the time between drug administration and testing are held constant. This time-dependence was not observed for the peripheral cholinesterase inhibitor neostigmine. Further validation of the hypothesis of changes in central cholinergic functioning following training will require a more direct examination of the activity of cholinergic neurons.

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