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Previous work from this laboratory has demonstrated a dose-related physostigmine (PH)-induced cholinesterase inhibition and dose-response performance decrements in exercising rats. In the present work, the optimal dose of atropine (AT) and scopolamine (S) as adjuncts to PH administration to attenuate the PH-induced performance decrements were determined. Experimental rats received via tail vein: 100, 200, or 400 ug/kg of AT prior to 200 ug/kg of PH or 4, 8, 16, or 32 ug/kg of S prior to 200 ug/kg of PH; controls received 2 saline injections. Fifteen min after drug administration, rats were run to exhaustion on a motor driven treadmill (11 m/min, 6° incline, 26°C, 50% rh;. Run time and heating rate (rate of rise of core temperature) for the AT experiment (as % of saline

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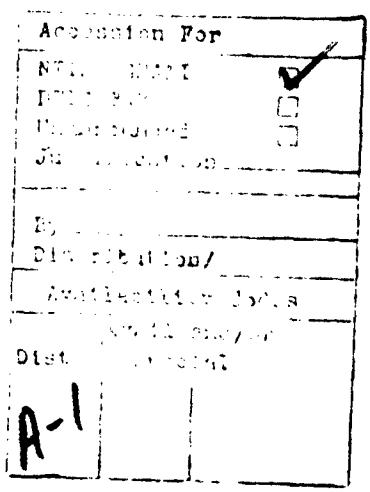
19. Abstract cont.

control values) were: saline+PH- 48, 214%; 100 ug/kg AT+PH- 52, 176%; 200 ug/kg AT+PH- 70, 157%; 400 ug/kg AT+PH- 48, 201%. For the S experiment run times and heating rates were: saline +PH- 57, 170%; 4 ug/kg S+PH- 70, 155%; 8 ug/kg S+PH- 82, 126%; 16 ug/kg S+PH- 77, 144%, and 32 ug/kg S+PH- 77, 157%. These data indicate that 200 ug/kg of AT or 8-16 ug/kg of S are the optimal doses of each drug for use as adjuncts to relieve the decremental effects of PH in exercising rats. Earlier work from this laboratory using sedentary heat-stressed rats has shown that the muscarinic cholinergic potency of S is 16 times that of AT; therefore, the optimal 200 ug/kg dose of AT would be approximately equivalent to 12.5 ug/kg of S. Since these values agree well with those determined in this experiment, the improvement in performance seen with AT and S adjuncts to PH may be primarily due to muscarinic cholinergic blockade.



ATROPINE AND SCOPOLAMINE AS ADJUNCTS TO PHYSOSTIGMINE IN EXERCISING RATS

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ABSTRACT

Previous work from this laboratory has demonstrated a dose-related physostigmine (PH)-induced cholinesterase inhibition and dose-response performance decrements in exercising rats. In the present work, the optimal dose of atropine (AT) and scopolamine (S) as adjuncts to PH administration to attenuate the PH-induced performance decrements were determined. Experimental rats received via tail vein: 100, 200, or 400 ug/kg of AT prior to 200 ug/kg of PH or 4, 8, 16, or 32 ug/kg of S prior to 200 ug/kg of PH; controls received 2 saline injections. Fifteen min after drug administration, rats were run to exhaustion on a motor driven treadmill (11 m/min, 6° incline, 26°C, 50% rh). Run time and heating rate (rate of rise of core temperature) for the AT experiment (as % of saline control values) were: saline+PH- 48, 214%; 100 ug/kg AT+PH- 52, 176%; 200 ug/kg AT+PH- 70, 157%; 400 ug/kg AT+PH- 48, 201%. For the S experiment run times and heating rates were: saline +PH- 57, 170%; 4 ug/kg S+PH- 70, 155%; 8 ug/kg S+PH- 82, 126%; 16 ug/kg S+PH- 77, 144%; and 32 ug/kg S+PH- 77, 157%. These data indicate that 200 ug/kg of AT or 8-16 ug/kg of S are the optimal doses of each drug for use as adjuncts to relieve the decremental effects of PH in exercising rats. Earlier work from this laboratory using sedentary heat-stressed rats has shown that the muscarinic cholinergic potency of S is 16 times that of AT; therefore, the optimal 200 ug/kg dose of AT would be approximately equivalent to 12.5 ug/kg of S. Since these values agree well with those determined in this experiment, the improvement in performance seen with AT and S adjuncts to PH may be primarily due to muscarinic cholinergic blockade.

INTRODUCTION Carbamates are anticholinesterases which reversibly bind acetylcholinesterase (AChE) and protect the enzyme from being irreversibly bound by organophosphates (OP) (2). Pyridostigmine (PY), a quaternary compound which does not cross the blood-brain barrier (peripherally acting), is currently being fielded as a prophylactic treatment against OP (5). Physostigmine (PH) is a tertiary compound that does penetrate the blood-brain barrier (centrally and peripherally acting) (2) thereby protecting AChE in the central nervous system. PH's central effects result in a superior protective value of PH over PY against a nerve agent challenge (3,5). However, PH administration can result in undesirable side effects including: excessive bronchial, salivary, and sweat secretions, fasciculations of voluntary muscles, increased intestinal motility, decreased endurance, and behavioral deficits (2,5,8).

In a running rat model, PH administration results in a dose-dependent decrease in endurance and an increase in rate of rise of core temperature (6). Many of the undesirable effects of PH could be counteracted by simultaneous administration of an anticholinergic (2). This study evaluates atropine and scopolamine as candidate adjuncts to PH.

Atropine (AT), a commonly used clinical drug, is the prototype of cholinergic mucsarinic blocking agents (2) and, as such, is a standard of comparison for other anticholinergic drugs. AT is used as a treatment drug for OP poisoning and has been shown, in combination with PH, to be efficacious as a prophylaxis against poisoning by OP's (4).

Scopolamine (S) is also an anticholinergic drug with more potent antimuscarinic properties than those of AT (2,7); it is also used as an anti-motion sickness drug (2) and is being evaluated as a second generation anticholinergic.

METHODS Adult male Sprague-Dawley rats (Charles River, CD strain, 510-530g, N=12/group) were used one time only. The animals were housed in an environmental chamber maintained at 26°C and 50% rh. Physostigmine salicylate, atropine sulfate, and scopolamine hydrobromide were all provided by Walter Reed Army Institute of Research; each of these drugs was dissolved in 0.2 ml of sterile normal saline and injected via tail vein.

Each animal received 2 injections 10 min apart. The first was saline or the appropriate dose of the anticholinergic drug followed by a second injection of saline or 200 ug/kg of physostigmine salicylate (60% inhibition of whole blood cholinesterase (6)). A range of doses of AT and S were used in order to determine an optimum dose. This study did not examine the effects of the anticholinergics alone on run performance; that data can be found in a companion paper (1) and in an earlier publication (8). The doses were 100, 200, and 400 ug/kg of freebase AT as the sulfate for the AT+PH experiments and 4, 8, 16, 32 ug/kg of freebase S as the hydrobromide for the S+PH experiments.

Following PH injection the animals were monitored for cholinergic and drug side-effects (tremors, salivation,

exophthalmus, defecation, and overall activity) then weighed and instrumented with thermistors to measure core and tail skin temperatures. All rats were then run (11 m/min, 6° incline, 26°C, 50% rh) on a motor driven treadmill with a shock avoidance

FIG 1

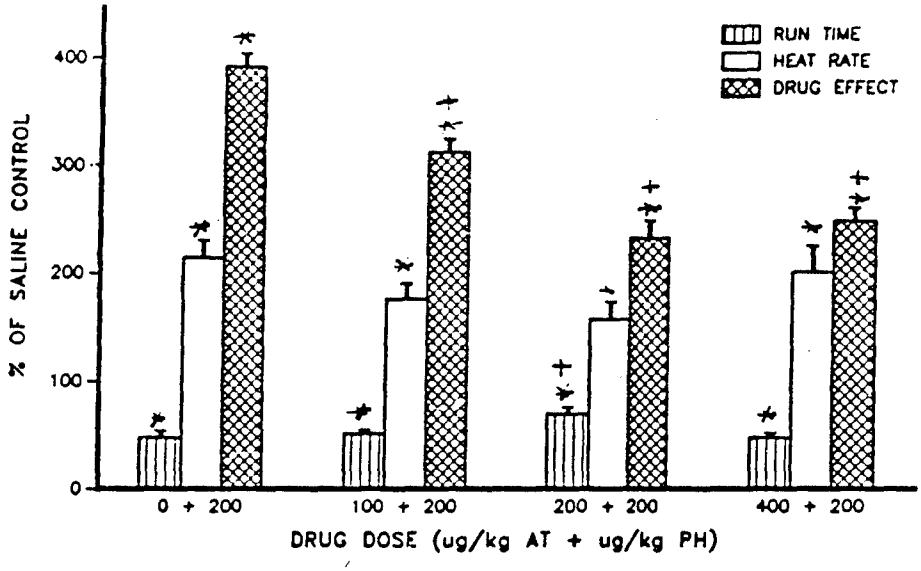

FIG 2

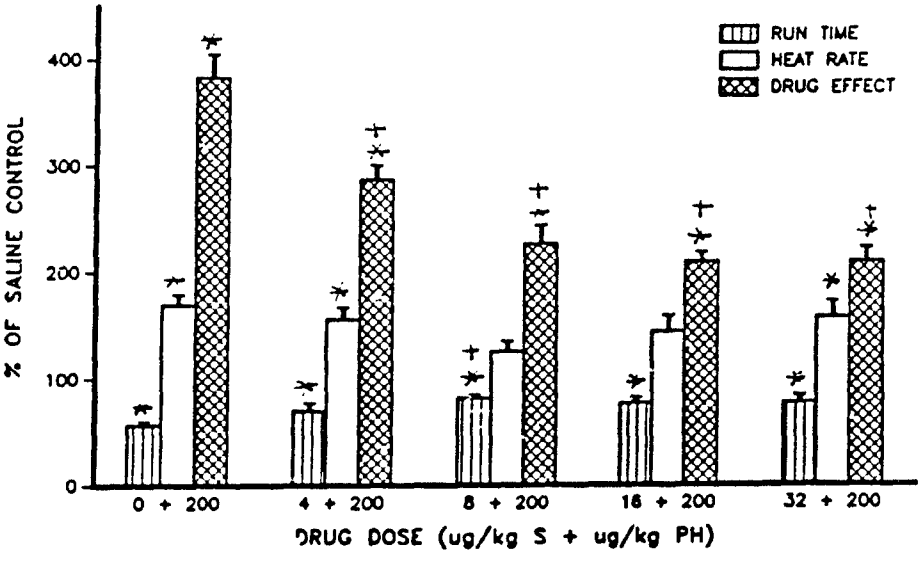

Fig 1 AT + PH in the running rat. Fig 2 S + PH in the running rat. A star (*) indicates that the value is significantly different from saline controls; + indicates that the value is significantly different from the values of the PH group.

contingency to exhaustion (unable to maintain the pace or right themselves when placed on their backs).

Statistical significance ($p<.05$) was determined by a one-way analysis of variance followed by Student-Newman-Keuls multiple range test for all pair comparisons.

RESULTS In Fig 1 run time (endurance), heating rate (rate of rise of core temperature), and drug effect (score on a cholinergic symptom and run performance check list) are all plotted as a % of saline control values for the groups in the AT experiment. Note that all of the values in Fig 1 are different from controls; therefore, no dose of AT completely restored PH induced endurance or thermoregulatory decrements. All doses of AT improved the drug effect score, and 200 ug/kg improved endurance. Note that increasing the AT from 200 to 400 ug/kg increased heating rate and decreased endurance. Therefore, 200 ug/kg AT (equivalent to a 2 mg dose in man (7)) is the optimum dose as an adjunct to PH.

Fig 2 illustrates the results of the S experiment; the variables and symbols are as described for Fig 1. Although all groups with S showed an improvement in endurance over the group with PH alone, only the 8 ug/kg S group had a run time that was significantly greater than that of the PH group. The 8 and 16 ug/kg S groups had heating rates that were not significantly different from those of the control group. The high heating rate of the 4 ug/kg group indicates that this is insufficient S, and the elevated heating rate of the group with 32 ug/kg indicates that this is excessive S. All the S-treated groups had drug effect scores that were significantly better than that of the PH group. These results suggest that 8-16 ug/kg of S (equivalent to a 80-160 ug dose in man (7)) is the optimum dose to be used as an adjunct to PH.

CONCLUSION Earlier work from this laboratory using sedentary heat-stressed rats has shown that the muscarinic cholinergic potency of S is 16 times that of AT (8); therefore, the optimal dose of AT (200 ug/kg) would be approximately equivalent to 12.5 ug/kg of S. These values agree very well with the 200 ug/kg of AT and the 8-16 ug/kg of S determined above; thus, the improvement in performance seen with AT and S as adjuncts to PH in the exercising rat may be primarily due to muscarinic cholinergic blockade.

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