Anticholinergics: Effects on Thermoregulation and Performance in Rats

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MATTIEOUTH, C. B. Anticholinergics. Effects on thermoregulation and performance in rats. NEUROSCI BIOBEHAV REV 15(4) 141-146, 1991 — Atropine (AT) induces a dose-dependent increase in rate of rise of core temperature (heating rate) in sedentary heat-stressed rats, a muscarinic anticholinergic (MA) effect which is quantitatively similar to the increase in heating rate seen in heat-exposed men after equivalent atropine dose. In the heat-stressed rat, scopolamine (S) was found to have 16 X the MA effect of AT and, in the present study, aprophen (AP) and trihexyphenidyl (THP) manifested 0.067 X and 0.061 X the MA effect of AT. In rats exercising on a treadmill (11 m/min, 6° incline, 26°C), physostigmine (PH) administration resulted in reduced endurance and increased heating rate, both of which were attenuated following AT administration — hypothesized to be a nicotinic anticholinergic (NA) effect. Optimum doses of anticholinergics to reverse the PH-induced decrements were AT ~ 200 μg/kg, S ~ 8-16 μg/kg, AP ~ 3000 μg/kg (MA), and THP ~ 800 μg/kg. These optimum NA doses for AT, S, and AP were the same as those predicted from their MA potency relative to AT in heat-stressed rats. However, it should be noted that 800 μg/kg of THP is only ¼ of the expected 3200 μg/kg dose of THP based on MA equivalence to AT. Relative MA activities and optimum doses in PH-treated exercising rats appear to be due to differential MA and NA activities. Thus, a combination of both sedentary heat-stressed and exercising rat models may be useful in predicting relative cholinergic effects of new drugs with both MA and NA effects in man.

Anticholinergic Anticholinesterase Rat Model Nicotinic Muscarinic Exercise Temperature regulation Hyperthermia

THE effects of acetylcholine can be categorized as muscarinic or nicotinic depending on the type of receptor activated. Stimulation of muscarinic receptors results in, generalized vasodilation, decreased heart rate and cardiac contractility, increased secretion of all exocrine glands, including sweat and salivary, increased intestinal and gastric contractions, and increased gastric and tracheobronchial secretions (14). Low to moderate doses of an agonist at nicotinic receptors result in stimulation of autonomic ganglia and stimulation at neuromuscular junctions, at high doses receptor blockade and muscle paralysis may result (14). Over stimulation at the neuromuscular junction (nicotinic) results in fasciculations, asynchronous excitation, fatiguability, and involuntary twitching (14). The fasciculations and involuntary twitching result in increased metabolic activity and consequent increased heat production.

We have previously reported that atropine (AT, the prototype of muscarinic anticholinergic drugs) induces a dose-response increase in heating rate (rate of rise of core temperature) in the sedentary heat-stressed rat (18,25). This is a muscarinic anticholinergic (MA) effect, because it results from inhibition of salivation. In hot environments rats spread saliva over the vermil surface of their bodies for evaporative cooling (16). Both saliva secretion in rodents (18,25) and sweat secretion in humans (6, 20, 21) are inhibited by atropine. Clubley et al. (5) simultaneously quaternary carbamate anticholinesterase atropine. A 2 mg dose of AT will suppress the sweat rate of humans in the heat by about 40% (7, 8, 30, 31). In the rat, 200 μg/kg is equivalent to 2 mg in man (12), and 250 μg/kg of AT elicits a similar 90% inhibition of water loss via saliva in the rat (18,25). Additionally, the time course of action of AT in the rat closely approximates that seen in humans (25).

Administration of the anticholinesterase physostigmine (PH, 200 μg/kg) to rats exercising on a treadmill, at a dose resulting in an increased heating rate and a decreased endurance (23,26), is accompanied by marked fasciculations and involuntary twitching (nicotinic) (14,26). The decreased endurance could be due to nicotinic-induced over stimulation and fatiguability of the running muscles. The increased heating rate of rats exercising after PH administration is related to the muscular activity of running, because PH administration to sedentary rats does not increase core temperature (24,28,29). In fact, the tail vasodilation (muscarinic) evident with PH administration, results in decreased heat storage in sedentary rats (26,29). Tail vasodilation dissipates the increased metabolic heat generated in sedentary PH-treated rats but is inadequate for heat dissipation in the exercising rats (26). Further, the increased heating rate in exercising rats appears to be a peripheral effect, since the same increased heating rate results from the administration of pyridostigmine, a quaternary carbamate lacking in central effects at normal dose levels (27). When a rat is running on a treadmill, an increase in salivary water loss (muscarinic) is of no thermoregulatory bene-
fit, because it can not be spread behaviorally. Thus, running rats can dissipate heat only through dilation of tail vessels (29). While the administration of PH should theoretically result in an increased concentration of acetylcholine at all receptors (32), we hypothesize that the decrements in performance of PH-treated exercising rats are due to nicotinic stimulation. If there is improvement in endurance or thermoregulation of PH-treated exercising rats following the administration of anticholinergics, we propose that this is a nicotinic anticholinergic (NA) effect of the drugs.

This study examines the effects of 4 anticholinergic drugs in the sedentary heat-stressed rat and in the exercising PH-treated rat to determine their relative MA and NA potencies. These 4 drugs (atropine, scopolamine, aprophen, and trihexyphenidyl) are being considered, because they have all been proposed as adjuncts to ameliorate side effects induced by PH administration (3, 9, 11, 19, 23, 26).

**Method**

**Animals**

Adult male Sprague-Dawley rats (Charles River, CD strain, 510-530 g, N=12/group) were caged individually in wire-bottomed cages in an environmental chamber (4 x 3 x 2 m) at 26°C and 50% rh, and used one time only. Lighting was controlled automatically (on, 0600-1800 h) and Purina rat chow and water were available ad lib except during experimental intervals.

**Drugs**

Aprophen hydrochloride (AP) (Starks Associates, Inc., Buffalo, NY 14213, Lot No. JF14-53-4), atropine sulfate (AT) (Sigma Chemical Company, St. Louis, MO 63178, Lot No. 35F-0648), scopolamine hydrobromide (S) (Boehringer Ingelheim, West Germany, no Lot No.), physostigmine salicylate (PH) (Sandoz, Wander, Roussel Corp. NY, Lot No. 85003.01), and trihexyphenidyl hydrochloride (THP) (Sigma Chemical Co., Lot No. 2F-5098) were dissolved in 0.2 ml of sterile 0.9% saline and administered via lateral tail vein (IV). All anticholinergic drug doses are calculated as the free base, but the physostigmine dose is calculated as µg/kg of the salicylate. To get rat equivalents to human clinical drug dose ranges, the dose/kg in man was multiplied by 7 (12).

**Sedentary Heat Stress Procedure**

To determine the anticholinergic potencies of AP and THP relative to that of AT, a dose within the equivalent clinical human dose range (14) of each was administered IV 15 min prior to heat stress. Unrestrained rats were heat stressed in their own cages in a 1 x 2 x 2 m chamber maintained at 41.5°C and 30% rh until a core temperature (Tc) of 42.6°C was attained. At that time the animals were removed from the heat, weighed, and allowed to cool passively in a 26°C chamber. During heating and cooling, weight loss (as a measure of fluid spread for evaporative cooling), corrected for fecal pellet production, and core temperature (rectal probe inserted 6.5 cm) were monitored. Using the heating rates of these animals, an anticholinergic potency relative to atropine was calculated as previously reported (24).

**Exercise Procedure**

To determine an optimum dose (the dose that comes as close as possible to returning endurance and thermoregulation to control levels) of each anticholinergic drug to be used as an adjunct to PH in the running rat, a range of doses of each of the 4 anticholinergic drugs (AT, AP, S, THP) were evaluated. The dose of each drug calculated to be equivalent to 200 µg/kg (0.69 µM/kg) of AT in the sedentary heat-stress experiments (Table 2) was the initial dose in the exercise studies. 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 µg/kg (0.48 µM/kg) of PH. We have previously reported that administration of this dose and form of PH resulted in 60% inhibition of whole blood cholinesterase (23). Fifteen minutes after drug administration the rats were run (motor driven treadmill, 11 m/min, 6° incline, 26°C, 50% rh, shock avoidance contingency) until exhausted. Exhaustion is defined as the point at which rats do not right themselves when placed on their backs.

**TABLE 1**

<table>
<thead>
<tr>
<th>DRUG EFFECT CHECK LIST FOR RUNNING RATS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tremors*</td>
</tr>
<tr>
<td>none</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>Salivation†</td>
</tr>
<tr>
<td>none</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>Exophthalmos‡</td>
</tr>
<tr>
<td>no</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>Defecation§</td>
</tr>
<tr>
<td>none</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>Run Performance¶</td>
</tr>
<tr>
<td>excellent</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>Overall Behavior#</td>
</tr>
<tr>
<td>normal</td>
</tr>
<tr>
<td>or under</td>
</tr>
<tr>
<td>0</td>
</tr>
</tbody>
</table>

* Moderate tremors (score of 2).
† Copious dripping saliva (score of 3).
‡ Slight to definite exophthalmos (score of 1-2).
§ Defecation in excess of 2 pellets (score of 1-3).
¶ Poor run performance or inability to run at all (score 2-3).
# Moderately hypoactive (lethargic, score of 2).
During the run and subsequent recovery, Tc (core temperature) and Tt (tail skin temperature) were monitored.

The administration of PH alone results in readily detectable side effects. A checklist (Table 1) was used to quantitate these side effects and to determine any improvement in these effects resulting from the administration of the 4 anticholinergics. The first 4 items were evaluated during the 15 min between the injection of PH and the start of run, and the last 2 were judgments based on performance during the run. All evaluations were made by an observer without knowledge of the treatment group.

Statistical Analysis

All values are mean ± SE. The data were analyzed by a one-way analysis of variance followed by the Student-Newman-Keuls multiple range test for all pair comparisons. The null hypothesis was rejected at the p < 0.05 level. Values in Figs. 1–4 and Table 3 are given in terms of % of saline controls in order to use only one y-axis for the 3 variables heat rate, run time, and drug effect. Significant differences among all groups were determined on the raw data that these %'s represent.

RESULTS

Sedentary Heat-Stressed Rats

Rate of rise of core temperature (heating rate) was found to be the most sensitive index of atropine effect in the sedentary heat-stressed rat (26). Therefore, the atropine-induced dose response increase in heating rate was used to calculate an anticholinergic potency of 16 for S relative to a value of 1 for atropine (Table 2) (24). Table 2 contains the heating rates for the indicated doses of AP and THP determined in this study. Using the method of Matthew et al. (24), these heating rates and doses were used to calculate anticholinergic potency ratios of 0.067 and 0.061 for AP and THP, respectively, relative to a value of 1 for AT. As an alternative means of comparing these 4 drugs, the 200 μg/kg dose of AT was divided by the potency ratio of each drug to determine the dose of each drug that would theoretically inhibit thermoregulation in the heat-stressed rat to the same extent as 200 μg/kg of AT. If there is a dose-response relationship between drug dose and heating rate for S, AP, and THP as has been demonstrated for AT in the sedentary heat-stressed rat (25), the administration of 12 μg/kg of S, 3000 μg/kg of AP, or 3200 μg/kg of THP should elicit a heating rate of 0.08°C/min.

Performance Data for Exercising Rats

Figures 1–4 illustrate the results of experiments to determine the dose of each of the 4 anticholinergic drugs tested that optimize PH-decremented endurance and thermoregulation in exercising rats. In each of these figures run time (endurance), heating rate, and drug effect (score on a cholinergic symptom and run performance check list, Table 1) are plotted as a % of saline control values. Separate control and PH alone groups were done for each trial; there were no significant differences among these 4 control groups or among the 4 PH groups. The mean values for run time, heating rate, and drug effect for controls (C) and PH groups across the 4 sets of studies were C— 62 ± 6 min, 0.052 ± 0.007 °C/min, and 2.3 ± 0.2; PH— 33 ± 4 min, 0.096 ± 0.008°C/min, and 8.9 ± 0.9.

In Fig. 1 note that all of the values are significantly different from controls; therefore, no dose of AT completely restored PH-induced endurance and thermoregulatory decrements. While, all doses of AT significantly improved the drug effect score, only the 200 μg/kg dose of AT significantly improved endurance (run time) over that of the PH group. Increasing the AT from 200 to 400 μg/kg increased heating rate and decreased endurance. Therefore, 200 μg/kg AT [equivalent to a 2 mg dose in man (12)] is the optimum dose as an adjunct to PH.

TABLE 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (μg/kg)</th>
<th>Heating Rate (°C/min)</th>
<th>Potency Ratio*</th>
<th>AT = Dose† (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>—</td>
<td>0.022 ± 0.001</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Atropine</td>
<td>200</td>
<td>0.079 ± 0.004</td>
<td>14</td>
<td>200</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>(0.03)</td>
<td>0.079 ± 0.005</td>
<td>16‡</td>
<td>12</td>
</tr>
<tr>
<td>Aprophen</td>
<td>2000</td>
<td>0.072 ± 0.007</td>
<td>0.067</td>
<td>3000</td>
</tr>
<tr>
<td>Trihexyphenidyl</td>
<td>400</td>
<td>0.033 ± 0.004</td>
<td>0.061</td>
<td>3200</td>
</tr>
</tbody>
</table>

*Calculated muscarinic potency relative to atropine (24) corrected for differences in molecular weight.

†Atropine equivalent dose = 200 μg/kg AT/potency ratio. This dose of each drug should elicit the same heating rate in the heat-stressed rat as 200 μg/kg of AT.

‡Values (mean ± SE) taken from Matthew et al. (24). The saline group-heating rate was reconfirmed in this experiment.
FIG. 2. Run time, heating rate, and drug effect score plotted as a % of saline control values for rats given 200 μg/kg of PH plus the indicated dose of S prior to exercise. Significant (p<0.05) difference from saline controls is indicated by a C and from the group given PH alone by PH.

80-160 μg dose in man (12)] is the optimum dose to be used as an adjunct to PH.

In Fig. 3 it can be observed that all doses of AP significantly improved the drug effects induced by PH administration, and while all doses of AP increased endurance slightly over that of the PH group, none was significantly greater. AP (3 mg/kg) did attenuate the heating rate of PH pretreatment. The group receiving 6 mg/kg of AP had a shorter run time, higher heating rate and greater drug effect score than the group receiving 3 mg/kg, and the 3 mg/kg group had the lowest heating rate and the lowest drug effect score of all the groups receiving both drugs. Therefore, we concluded that 3 mg/kg [equivalent to a 30 mg dose in man (12)] should be the optimum dose when used as an adjunct to PH in the exercising rat.

All three groups receiving both THP and PH (Fig. 4) had drug effect scores that were significantly lower than that of the PH group but also higher than the controls, the 800 μg/kg group had a score closest to that of the control group. Run time was significantly lower than control in all 3 groups receiving both drugs, but the group receiving 800 μg/kg THP had a run time that was significantly greater than that of the PH group. The heating rates of the 400 and 1200 μg/kg groups were greater than those of the control group and not different from the PH group, however, the 800 μg/kg group had a heating rate that was significantly lower than that of the PH group and not different from that of controls. From these data it appears that the 800 μg/kg dose of THP [equivalent to 8 mg/70 kg man (12)] is the optimum dose as an adjunct to PH in the running rat.

Table 3 summarizes the results of these 4 experiments. The optimum dose of each drug as an adjunct to PH pretreatment in exercising rats as suggested by our data is noted in the first column. Note that except for THP these are the same values as the MA equivalent doses tabulated in the last column of Table 2. A human dosage, equivalent to the optimum rat dose, was calculated by dividing the rat dose by 7 (12) and then multiplying by 70 kg/adult to get an adult dose. These doses all fall within or below the clinically used range. All of the adjuncts decreased the PH elevated heating rate, but both THP and AP elicited significantly lower heating rates than AT. The run time as a % of control values indicates that the optimum dose of each adjunct increased run time to the same extent. Additionally, the optimum dose of THP in running rats is only 1/4 the AT equivalent dose for THP in the sedentary heat-stressed rat.

DISCUSSION

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 (23). Many of the undesirable effects of PH could be counteracted by simultaneous administration of an anticholinergic (14). This study evaluated the relative abilities of 4 anticholinergic drugs with both central and peripheral sites of action (atropine, scopolamine, aprophen and trihexyphenidyl) to ameliorate these PH-induced decrements.

In the present work, 200 μg/kg of PH [previously shown to elicit a 60% inhibition of whole blood cholinesterase (23)] decremented endurance and increased heating rate, and 300 μg/kg of AT did not completely restore endurance to control level. In an other previous study (26), this same dose of PH elicited a 40%
TABLE 3

COMPARISON OF 4 ANTICHOLINERGIC DRUGS IN
PH-TREATED RUNNING RATS

<table>
<thead>
<tr>
<th>Drug*</th>
<th>R Dose† (µg/kg)</th>
<th>H=Dose‡ (µg/adult)</th>
<th>Clinical§ HR % C5</th>
<th>RT % C5 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine</td>
<td>200</td>
<td>2</td>
<td>0.5-10</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>(0.69)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scopolamine</td>
<td>8-16</td>
<td>0.08-0.16</td>
<td>0.6</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>(0.03-0.05)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aprophen</td>
<td>3000</td>
<td>30</td>
<td>— #</td>
<td>115**</td>
</tr>
<tr>
<td></td>
<td>(9.23)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trihexyphenidyl</td>
<td>800</td>
<td>8</td>
<td>1-15</td>
<td>111**</td>
</tr>
</tbody>
</table>

*Each drug group received the indicated drug plus 200 µg/kg (0.48
µM/kg) PH.
†Optimum dose of each drug when used with PH in the running rat.
‡Human (70 kg adult) equivalent dose = ½ the rat dose on a mg/kg
basis (12).
§Range of doses in clinical use (14).
#Aprophen is not used clinically in the United States.
**Values are significantly different (p<0.05) from AT group.

Inhibition of whole blood cholinesterase and 200 µg/kg of AT
completely restored endurance and thermoregulation. The source
of the PH in the earlier work was Antirium, an injectable solu-
tion, (Forest Pharmaceuticals, Inc., St. Louis, MO), however, in
the present study, a fresh solution was made up every day using
PH obtained from Sandoz. This difference in source and form of
PH could explain the greater levels of inhibition and decremented
performance in the present study.

Atropine, a commonly used drug, is the prototype of
cholinergic muscarinic blocking agents (14) and, as such, has
become a standard for comparison with other anticholinergic
agents. Aprophen has also been widely used as a treatment drug
for organophosphate (OP) poisoning and is an effective pretreatment
against poisoning by OP's when used in combination with PH
(10,17). Additionally, AT and PH have each been used clinically
to counteract clinically induced side effects (2).

Scopolamine is also primarily a muscarinic anticholinergic
drug; apparently, however, it has more potent antimuscarinic
properties than atropine (2,19), and S has also been used to
antagonize PH effects (19). The central antimuscarinic effects of
scopolamine make it valuable as an antimotion sickness drug
(14,33). Neither AT nor S have previously been shown to have
significant NA effects except at doses far in excess of those used
clinically (14).

Aprophen is not a drug in clinical use in the United States, but
it is used in other countries as both a pre- and posttreatment for
OP poisoning (15). This drug has antimuscarinic, antinicotinic,
anticonvulsant, and local anesthetic properties (22). The in vivo
MA properties of AP are ¼ as potent as those of AT (9), and it
has been shown to have a greater affinity for nicotinic receptors
than AT (1,9).

Alternatively, THP (Artane) has several unique properties un-
like the other anticholinergics tested in these experiments. For
example, the anticholinergic effect of THP on salivary and sweat
glands is less than that of AT or S (6,14). This is a potential
advantage because THP as an adjunct to PH should not inhibit
heat dissipation as much as AT or S (6,24). Although AT and
THP both have MA effects, AT blocks both M-1 (central, neu-
ronal) and M-2 (secretory, smooth muscle and cardiac) muscar-
inic receptors, but THP has a greater specific affinity for the M-1
receptors than for either the secretory or cardiac receptors (4,13).
Thus, the tachycardia associated with AT administration might be
reduced with THP administration (4).

In the running rat model the optimum doses (Table 3) of AT
(200 µg/kg), S (8-16 µg/kg), and AP (3000 µg/kg) for use as
adjuncts to PH administration (NA) were precisely the doses
equivalent to 200 µg/kg of AT in the sedentary heat-stressed rat
(Table 2, MA). But, the optimum dose of THP in the running rat
(Table 3, 800 µg/kg) is only ¼ of the dose equivalent to 200
µg/kg of AT in the heat-stressed rat (Table 2, 3200 µg/kg). This
lower MA effect of THP relative to AT might be expected be-
cause of the M-1 specificity of THP. Hence, the optimum NA
do.se of THP may be far less detrimental to thermoregulation in
the heat than the optimum NA doses of AT, S, or AP.

Aprophen has been shown to have greater affinity for nicotinic
cholinergic receptors (greater NA effect) than AT (1,9), therefore
the demonstrated ability (Table 3) of AP over that of AT to re-
turn heating rates of exercising rats closer to control values was
expected. THP has been shown to antagonize the effects of PH-
induced accumulation of ACh (3,11), but the receptor site in-
volved has not been identified. On the basis of the present work
we suggest that if appropriate studies are done, it will be con-
cluded that THP has significant NA effects.

None of the 4 anticholinergic drugs used in this study has been
identified as a specific nicotinic antagonist. Therefore, we cannot
conclusively say that the PH-induced decrements in exercising
rats are nicotinic until another study using a specific nicotinic an-
ticholinergic drug such as d-tubocurarine, mecamylamine or hexa-
methonium has been done. However, the evidence presented here
suggests that the heat-stressed and exercising rat models may be
useful in determining relative in vivo MA and NA activities of
new drugs.

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not be construed as official Department of the Army position, policy or
decision, unless so designated by other official documentation. In con-
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with the Animal Welfare Act, and other Federal statutes and regulations
relating to animals and experiments involving animals and adhered to
principles stated in the Guide for the Care and Use of Laboratory Ani-
ma, NIH publication 86-23, 1985 edition. Further, all procedures
were reviewed and approved by the U.S. Army Research Institute of Envi-
ronmental Medicine's Animal Care and Use Committee. Citizens of com-
mmercial organizations and trade names do not constitute an official
Department of the Army endorsement or approval of the products or ser-
vices of these organizations.

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