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# Modulation of Cholinergic Activity and the Aversive Threshold in the Rat

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HOUSER, V. P. AND D. A. VAN HART. *Modulation of cholinergic activity and the aversive threshold in the rat.* PHARMAC. BIOCHEM. BEHAV. 2(5) 631–637, 1974. – The analgesic potency of atropine sulfate (5.0, 10.0, 20.0, 40.0 mg/kg), eserine sulfate (0.5, 1.0, 1.5 mg/kg), pilocarpine nitrate (5.0, 10.0, 20.0 mg/kg), scopolamine methylbromide (0.5, 1.0 mg/kg) and scopolamine hydrobromide (1.0 mg/kg) was measured in the rat using the spatial preference technique. Enhanced cholinergic tone via the administration of eserine or pilocarpine in conjunction with scopolamine methylbromide produced significant increments in the aversive threshold. These increments could not be accounted for solely by changes in motor activity or the debilitating effects of enhanced peripheral cholinergic stimulation. None of the anticholinergics tested affected the aversive threshold. Scopolamine hydrobromide (1.0 mg/kg), however, was able to fully block the increments in the aversive threshold noted after the administration of pilocarpine (10.0 mg/kg). These results were interpreted to suggest that agents which enhance cholinergic tone can produce significant analgesia in the rat. While no firm conclusions can be made without further evidence, especially with regard to the antianalgesic effects of the anticholinergics, it is possible that central cholinergic mechanisms may mediate the aversive qualities of electric shock in the rat.

Atropine sulfate  Eserine sulfate  Scopolamine methylbromide  Pilocarpine nitrate  Aversive thresholds

MODULATION of cholinergic tone via the introduction of various drugs has been reported to influence a variety of behaviors under aversive control. For example, cholinergic stimulants (i.e., arecoline, eserine, and pilocarpine) were able to inhibit the performance of a previously acquired conditioned avoidance response in rats [20]. Anticholinergic agents, on the other hand, produce a variety of effects depending on the dose administered and the species employed. Low doses of scopolamine (0.1 mg/kg) have been reported to enhance the acquisition of Sidman avoidance behavior in rats by increasing response rates and reducing shock rates [17,18]. Doses above 0.4 mg/kg, however, lead to a disruption of Sidman avoidance behavior, thus producing higher shock rates [8]. Squirrel monkeys have also been reported [9,11] to display impaired Sidman avoidance behavior in response to scopolamine administered in a wide range of doses (i.e., 0.06–1.0 mg/kg).

In attempting to account for the behavioral effects of some of these drugs, notably the anticholinergics, in aversive test procedures, some investigators [17,18] have suggested that these agents may alter the sensory characteristics of electric shock. Thus, the analgesic or antianalgesic effects of the anticholinergics, as well as the cholinergic stimulants, could account for some of their effects on behavior controlled by aversive schedules of reinforcement.

In order to measure the analgesic properties of various agents our laboratory has modified a technique first introduced by Campbell [1] which uses a spatial preference cage to determine the aversive threshold of rats to grid shock. This technique allows animals to escape various shock intensities by simply crossing from one side of the cage to the other. Previous reports from this laboratory have indicated that this technique is an extremely reliable and sensitive measure of drug-induced analgesia produced by a wide variety of analgesic agents known to be clinically active in man. For example, the technique is sensitive to a number of weak analgesics (i.e., sodium salicylate, indomethacin [14]), narcotic antagonist analgesics (i.e., pentozocine, cyclazocine [13]), as well as the classic narcotic analgesics (i.e., morphine [12], codeine, and meperidine hydrochloride [13]). The procedure also appears to be selective in that sedative doses of sodium pentobarbital which have been reported to be nonanalgesic in man [5] are also inactive in the spatial preference technique [13].

The above evidence suggests that the spatial preference technique may have several advantages over previous pharmacological tests used to determine drug-induced analgesia. Unlike such animal models as the tail-flick or hot-plate procedures, the spatial preference technique can detect analgesia in all three major classes of analgesic agents known to be active in man [13,14]. Furthermore, this technique appears to be somewhat selective in that it does not react to sedative doses of sodium pentobarbital [13]. Thus, the spatial preference technique may be superior to such analgesic assays as writhing induced by chemicals

which lack this selectivity and thus react to a host of agents which are clinically nonanalgesic in man [22]. Use of the spatial preference technique, therefore, appears to give the investigator a reasonably sensitive and selective measure of drug-induced analgesia.

The present report is an attempt to extend earlier work [15] which indicated that cholinergic stimulation via the administration of pilocarpine nitrate significantly elevated the aversive threshold, while the central acting anticholinergic, scopolamine hydrobromide, had no significant analgesic effects. To explore whether or not the cholinergic properties of pilocarpine were responsible for these effects the anticholinesterase, eserine sulfate, and another anticholinergic, atropine sulfate, were administered to rats subjected to the spatial preference test. Furthermore, to determine whether the peripheral parasympathomimetic effects of pilocarpine or its central effects could account for the previous results a peripheral acting anticholinergic, scopolamine methylbromide, as well as a central acting agent, scopolamine hydrobromide, were administered to rats prior to pilocarpine injections. If the peripheral effects of pilocarpine were responsible for the increased aversive thresholds, pretreatment with scoploamine methylbromide should abolish the analgetic response to pilocarpine. If, on the other hand, pilocarpine exerts its effects through the central nervous system, only scopolamine hydrobromide would be effective in blocking the effects of pilocarpine on the aversive threshold.

## METHOD

### *Animals*

Eighteen male Sprague-Dawley derived rats obtained from ARS/Sprague-Dawley, Madison, Wisconsin, were used in the present study. They weighed 274–346 g at the beginning of the experiment.

### *Apparatus*

The test chamber and apparatus have been described in detail elsewhere [12]. Briefly, the chamber consisted of a rectangular Plexiglas shuttlebox which was pivoted in the middle, allowing the box to tilt from side to side as the animal crossed from one end to the other. This tilting movement activated a light action Acro lever switch located at one end of the cage which controlled the presentation of shock. The stainless steel rods which formed the floor of the cage could be electrified by various intensities of shock (i.e., 30, 60, 90, 120, 150 $\mu$A). The shock stimulus was provided by a d.c. generator which produced a 60 Hz square wave output. This unit was designed specifically to provide a constant current across an animal even when resistance was altered radically due to an animal's movements [21]. Standard electromechanical scheduling and recording equipment was located in an adjacent room. It was used to automatically present the various shock intensities and to record the amount of time in seconds spent on the shock side of the cage for each intensity, as well as the number of crossing responses made during each shock intensity of the daily sessions.

### *Procedure*

Each animal was subjected to a 50-min experimental session, the same time each day, six days a week. An experimental session consists of five 10-min periods in which five separate current intensities (i.e., 30, 60, 90, 120, 150 $\mu$A) were presented in an ascending order. The shock was presented on one side of the cage for 5 min and then switched to the other side for the remaining 5 min of each current intensity. The animal could escape the shock side of the cage by merely crossing to the opposite or nonshock portion of the tilt cage. The shock was automatically switched from one side to the other every 5 min to insure that each animal sampled all shock intensities even if it failed to make a crossing response during the 10-min period that each intensity was presented. Each animal was treated at all five shock intensities every day. In order to control for possible position preference, the initial shock presentation on a particular day was alternated from one side to another in a random fashion.

The dependent measure consisted of the amount of time in seconds spent on the shock side of the cage for each shock intensity. The aversive threshold was calculated daily for each animal by determining the intensity of shock which an animal avoided 75% of the time. At subthreshold intensities the animal, by chance, would spend 50% of the time on the shock side of the cage. Since time spent on the shock side diminished as the shock intensity increased, the 75% threshold criteria required a simple interpolation process. If animals spent more than 25% of the available time on the shock side at the highest intensity (i.e., 150 $\mu$A), as was the case under some drug conditions, an aversive threshold could not be interpolated since no higher levels were presented. In these cases, a threshold value of 150 $\mu$A was arbitrarily assigned. The number of crossing responses made during each shock intensity was also recorded for each animal.

After ten sessions all animals demonstrated stable threshold values. Animals were then randomly assigned to three separate six-animal drug groups. Each drug was given in several separate doses in the following weekly series. Saline was administered for the first three days of each weekly series followed by three days of a particular drug dosage. Animals were not tested on the seventh day of these weekly series.

The five drugs administered in the present study consisted of pilocarpine nitrate (5.0, 10.0, 20.0 mg/kg), scopolamine methylbromide (0.5, 1.0 mg/kg), eserine sulfate (0.5, 1.0, 1.5 mg/kg), atropine sulfate (5.0, 10.0, 20.0, 40.0 mg/kg), and scopolamine hydrobromide (1.0 mg/kg). All drugs were dissolved in 0.9% saline and administered intraperitoneally in a volume of 1.0 ml/kg. The above drugs were administered 1/2-hr before threshold testing, with the exception of scopolamine methylbromide and scopolamine hydrobromide which were given 50 min before threshold testing. The first six-animal group received four doses of atropine sulfate during the first four weeks of the experiment followed by the various dosages of eserine sulfate during the final four weeks. The second group was administered scopolamine methylbromide during the first two weeks followed by treatment with this anticholinergic 20 min before administration of various doses of pilocarpine nitrate during the final three weeks of the experimental period. The third and final six-animal group received pilocarpine nitrate (10.0 mg/kg) during the first week of the experiment; scopolamine hydrobromide (1.0 mg/kg) during the second week; followed by pretreatment with scopolamine hydrobromide (1.0 mg/kg) 20 min before the administration of pilocarpine nitrate (10.0 mg/kg) during the third and final week of the experiment.

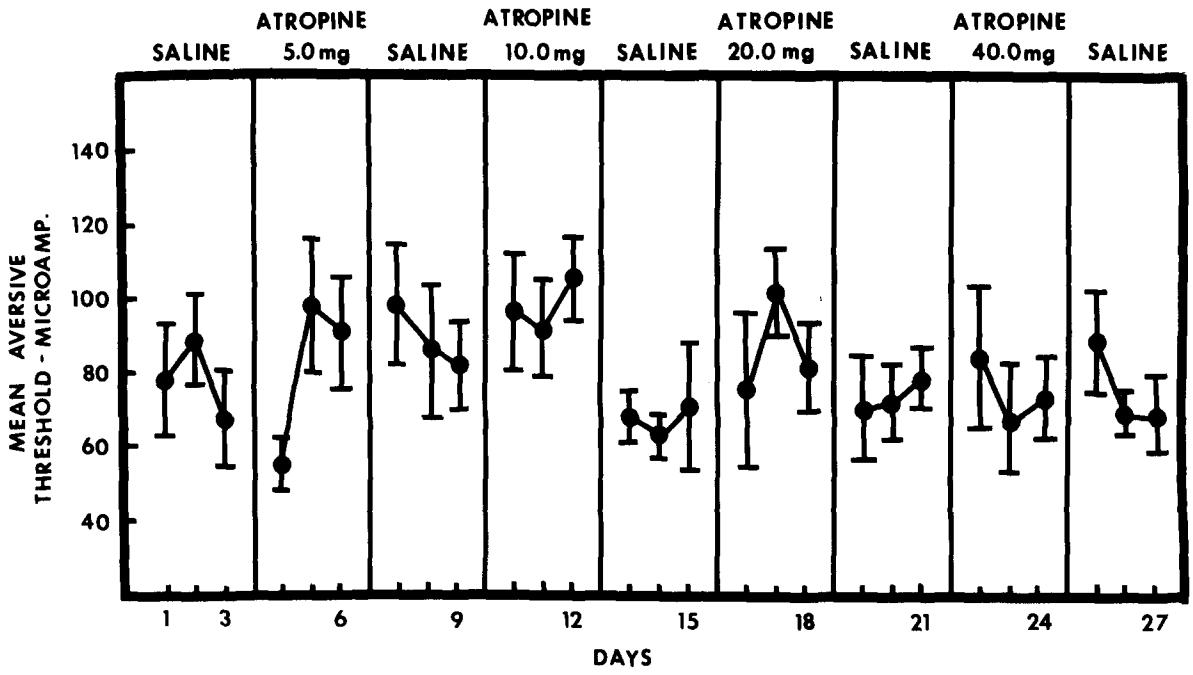


FIG. 1. Mean aversive thresholds with corresponding standard error of the means for 6 animals subjected to various doses (i.e., 5.0, 10.0, 20.0, 40.0 mg/kg) of atropine sulfate.

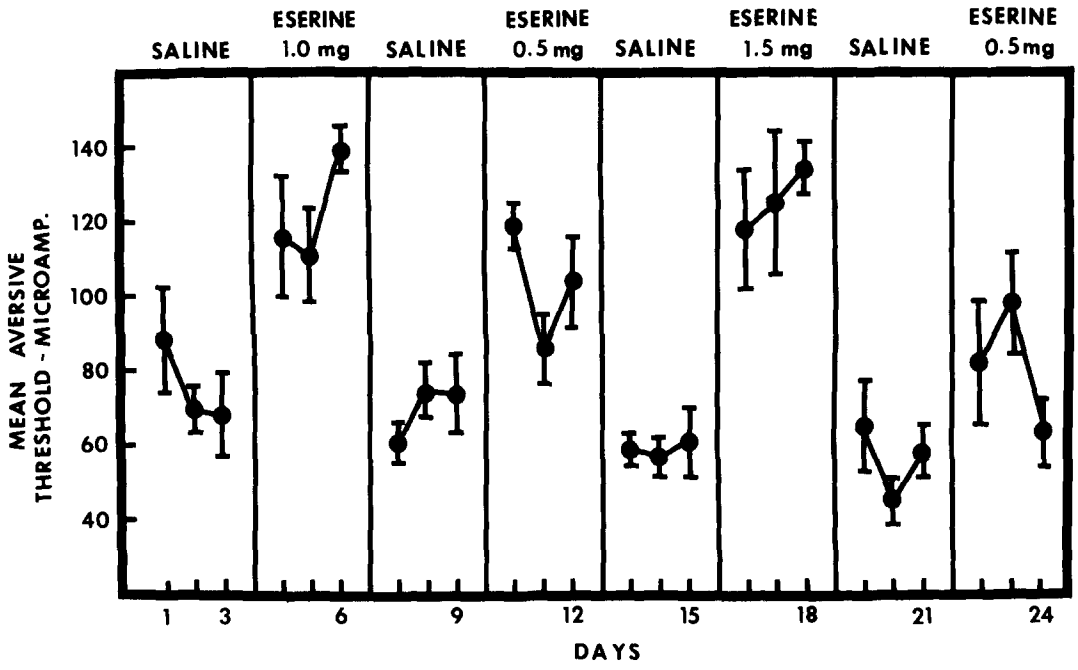


FIG. 2. Mean aversive thresholds with corresponding standard error of the means for 6 animals subjected to various doses (i.e., 0.5, 1.0, 1.5 mg/kg) of eserine sulfate.

## RESULTS

Figure 1 presents the mean aversive thresholds with corresponding standard error of the means for six animals subjected to various doses (i.e., 5.0, 10.0, 20.0, 40.0 mg/kg) of atropine sulfate.

These data indicate that atropine was not able to reliably affect the aversive threshold in any of the doses tested. A two-factor (within) analysis of variance [19] performed on the data indicated that none of the drug-saline comparisons reached significance ($p>0.05$), nor was there an overall main drug effect.

Figure 2 presents the mean aversive thresholds with

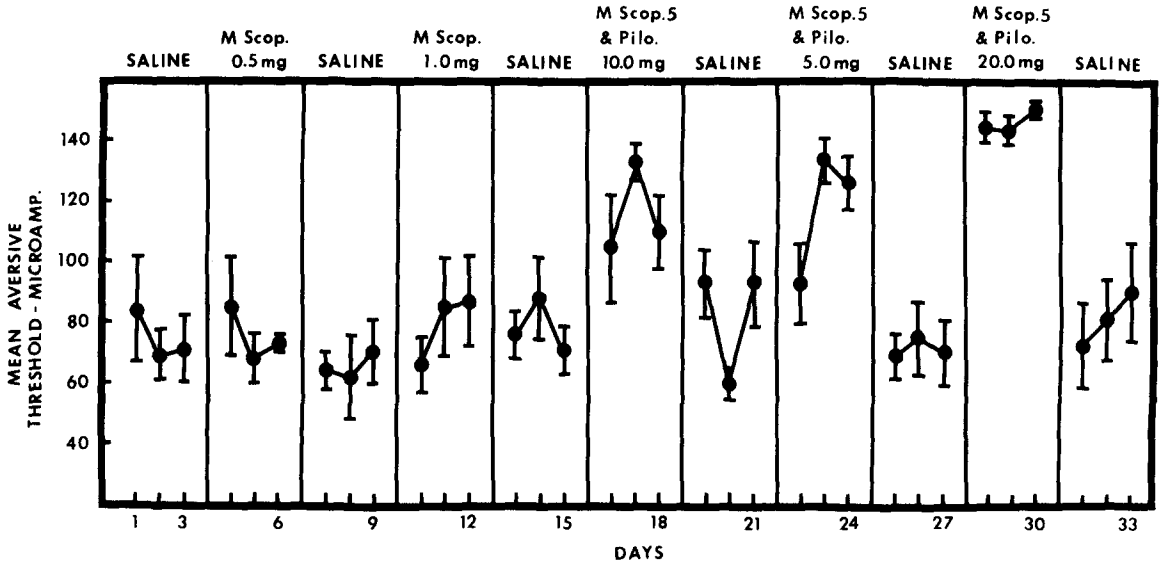


FIG. 3. Mean aversive thresholds with corresponding standard error of the means for 6 animals subjected to various doses of scopolamine methylbromide (i.e., 0.5, 1.0 mg/kg, 1/2-hr before testing) or pilocarpine nitrate (i.e., 5.0, 10.0, 20.0 mg/kg, 1/2-hr before testing) with a pretreatment of 0.5 mg/kg of scopolamine methylbromide (50 min before threshold testing).

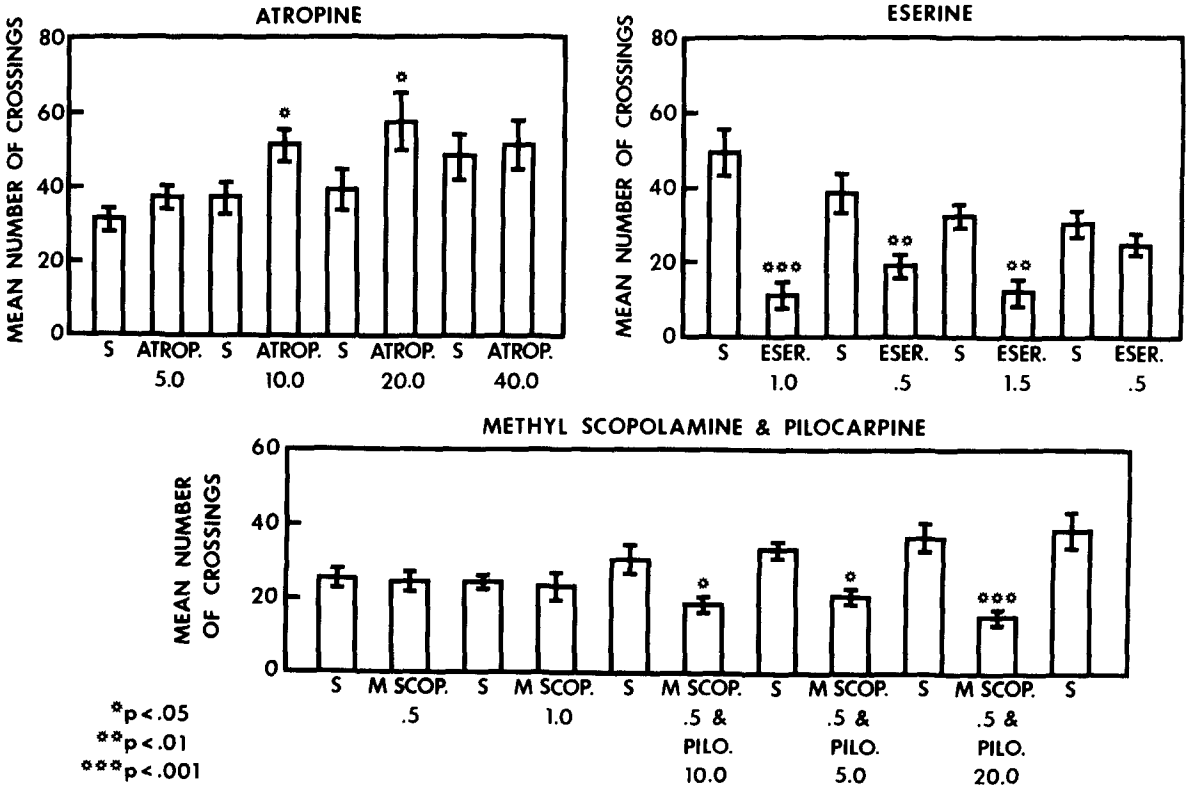


FIG. 4. Mean number of crossing responses with corresponding standard error of the means for each 6-animal group subjected to various doses of atropine sulfate, eserine sulfate, scopolamine methylbromide and/or pilocarpine nitrate. Each bar represents the mean of three consecutive drug or saline (S) sessions. All dosages are given in mg/kg.

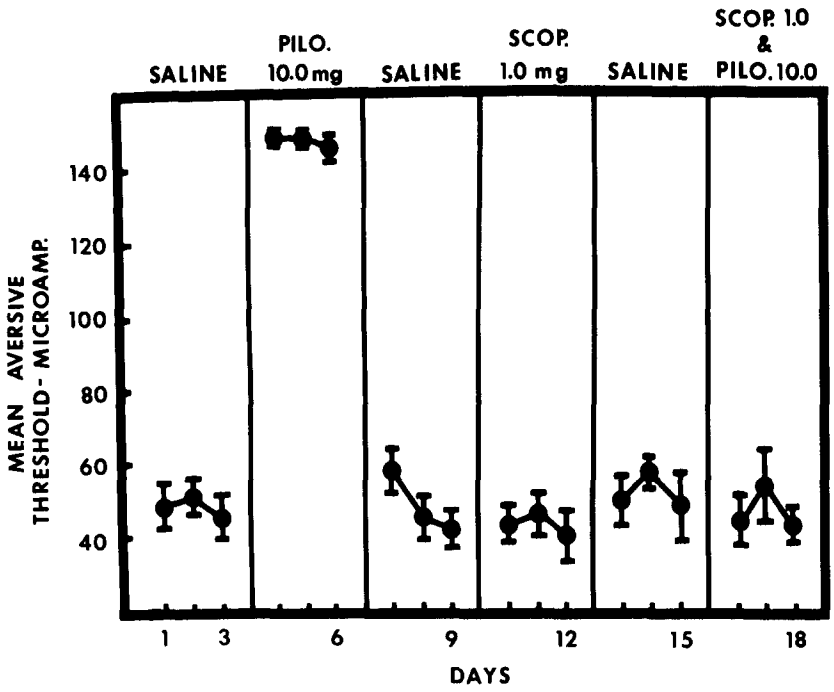


FIG. 5. Mean aversive thresholds with corresponding standard error of the means for 6 animals subjected to pilocarpine nitrate (10.0 mg/kg, 1/2-hr before testing); scopolamine hydrobromide (1.0 mg/kg, 1/2-hr before testing) or pilocarpine nitrate (10.0 mg/kg, 1/2-hr before testing) with a pretreatment of scopolamine hydrobromide (1.0 mg/kg, 50 min before threshold testing).

corresponding standard error of the means for six animals subjected to various doses of eserine sulfate (i.e., 0.5, 1.0, 1.5 mg/kg). The data in Fig. 2 clearly demonstrate that this particular anticholinesterase was able to raise the aversive threshold in a dose-dependent manner. A two-factor (within) analysis of variance [19] indicated that all the dosages tested significantly raised the aversive threshold ($p<0.025$). It should be noted that these elevations in the aversive threshold were dose related in that the higher dosages produced greater increments from the preceding saline values than the lower dosages.

Figure 3 presents the mean aversive threshold with corresponding standard error of the means for the six animals subjected to scopolamine methylbromide (i.e., 0.5, 1.0 mg/kg), given alone, or in conjunction with various doses of pilocarpine nitrate (i.e., 5.0, 10.0, 20.0 mg/kg). Figure 3 indicates that this peripheral acting anticholinergic was not able to raise the aversive threshold. Statistical comparisons using a two-factor analysis of variance further substantiated the lack of a significant increment in the aversive threshold in response to this drug ($p>0.05$). The administration of pilocarpine nitrate 20 min after scopolamine methylbromide, however, did produce significant increments in the aversive threshold ($p<0.005$) at all doses tested. Furthermore, the increments in the aversive threshold in response to pilocarpine administration appeared to be dose related with the highest dose producing a greater effect than the lower dosages.

Figure 4 presents the mean number of crossing responses with corresponding standard error of the means made while animals were subjected to various doses of atropine sulfate, eserine sulfate, scopolamine methylbromide and/or pilocarpine nitrate. Each bar represents the mean number of crossing responses made by six animals during three consecutive drug or saline sessions.

This figure indicates that although atropine had no significant effect on the aversive threshold, this anticholinergic was able to significantly ($p<0.05$) raise the number of crossing responses made during the middle range of doses (i.e., 10.0, 20.0, mg/kg). Eserine sulfate, on the other hand, significantly reduced motor activity ($p<0.01$) at all doses tested with the exception of the first replication of the 0.5 mg/kg dosage. Finally, scopolamine methylbromide had no effect on the number of crossings made when administered by itself. When this anticholinergic was given in conjunction with pilocarpine nitrate, however, motor activity showed significant decrements under all dosages of pilocarpine ($p<0.025$). It would thus appear that cholinergic stimulation via the administration of either pilocarpine or eserine leads to decrements in the number of crossing responses made in the spatial preference test.

Figure 5 presents the mean aversive threshold with corresponding standard error of the means for those animals subjected to scopolamine hydrobromide (1.0 mg/kg) and pilocarpine nitrate (10.0 mg/kg).

A two-factor (within) analysis of variance [19] indicated that pilocarpine nitrate (10.0 mg/kg), by itself, was able to produce reliable increments ($p<0.001$) in the aversive threshold. Scopolamine hydrobromide (1.0 mg/kg) administered alone had no effect on the aversive threshold and was able to completely block the effects of pilocarpine (10.0 mg/kg) when it was given 20 min before pilocarpine administration. An analysis of the crossing data indicated that pilocarpine (10.0 mg/kg) significantly reduced motor

activity ($p<0.01$), while scopolamine hydrobromide (1.0 mg/kg) given alone ($p<0.05$) or in conjunction with pilocarpine ($p<0.01$) significantly increased the number of crossing responses emitted by the animal subjects. Thus, a central acting anticholinergic (i.e., scopolamine hydrobromide) was able to block the pilocarpine-induced increment in the aversive threshold and was able to produce hyperactivity in animals treated with this cholinomimetic agent.

## DISCUSSION

The present results indicate that cholinergic stimulation via the administration of pilocarpine or eserine leads to reliable increments in the aversive threshold. These data are in agreement with a previous report from this laboratory [15] which demonstrated that pilocarpine, administered by itself, was able to reliably augment the aversive threshold in doses as low as 2.5 mg/kg. Since both eserine and pilocarpine given alone [15] or in conjunction with scopolamine methylbromide produce decrements in locomotor activity, it could be argued that the increments in the aversive threshold in response to these agents merely reflected the sedative properties of the two drugs employed. Several lines of evidence, however, argue against such an explanation. First, both pilocarpine [15] and eserine (e.g., Fig. 4) were able to produce reliable increments in the aversive threshold at dosages that did not significantly reduce motor activity. Secondly, an earlier report [15] has indicated that some anticholinergic agents in high doses (i.e., scopolamine hydrobromide, 2.0 mg/kg) can produce small reductions in the aversive threshold in conjunction with large statistically reliable decrements in motor activity. Thus, reductions in motor activity are not always correlated with increments in the aversive threshold. Furthermore, earlier work using the spatial preference technique [13] has indicated that a number of analgetic agents which are clinically active in man demonstrate clear dose-response relationships with regard to increments in the aversive threshold, combined with alterations in motor activity which may include: no change in activity, hyperactivity and/or hypoactivity in response to the same drug. For example, codeine sulfate was able to produce dose-related increments in the aversive threshold coupled with hyperactivity through the middle range of doses (15.0, 30.0 mg/kg) followed by a reduction in motor activity during the highest (i.e., 60.0 mg/kg) dosage [13]. Similar results were noted with morphine sulfate [12]. Cyclazocine, on the other hand, produced dose-related increments in the aversive threshold [13] in conjunction with no change in motor activity through the middle range of doses (i.e., 1.0, 2.0, 4.0 mg/kg) followed by hyperactivity under the highest dosage (i.e., 8.0 mg/kg). Similar results were noted with meperidine hydrochloride except that the highest dosage (40.0 mg/kg) produced significant activity decrements [13]. The above results clearly indicate that increments in the aversive threshold can occur in conjunction with: no change in motor activity, hyperactivity or hypoactivity. These findings, in turn, suggest that elevations in the aversive threshold are often independent of changes in motor activity. Thus, the increments in the aversive threshold noted in response to eserine and pilocarpine are not necessarily the result of decrements in motor activity. The fact, however, that significant reductions in activity did occur in conjunction with increased thresholds under pilocarpine and eserine does not allow one to completely rule out the possibility that cholinergic stimulants augment the aversive threshold through their sedative properties.

A comparison of the effects of pilocarpine, given alone (Fig. 5), and when it was administered after pretreatment with scopolamine methylbromide (Fig. 3) suggests that the peripheral acting anticholinergic may have attenuated the effects of pilocarpine somewhat. This comparison may be misleading, however, since the increments in the aversive threshold under 10.0 mg/kg of pilocarpine in Fig. 5 were greater than those previously recorded [15]. An earlier report [15] indicated that 10.0 mg/kg of pilocarpine given alone, raised the aversive threshold to a mean level of approximately 123 $\mu$A, while this same dosage of pilocarpine given after pretreatment with scopolamine methylbromide raised the threshold to approximately 117 $\mu$A. Thus, it would appear that the peripheral acting anticholinergic did not substantially attenuate the effects of pilocarpine on the aversive threshold. The large increments in response to 10.0 mg/kg of pilocarpine noted in Fig. 5 are difficult to explain, but could be a reflection of the fact that this dosage series represented the first time that these animals had been subjected to a drug, unlike the animals in the earlier report [15] who had received some experience with lower dosages of pilocarpine before the 10.0 mg/kg dose was administered.

The fact that scopolamine methylbromide could not block the actions of pilocarpine demonstrates that the peripheral parasympathomimetic effects of pilocarpine (i.e., diarrhea, excessive salivation, miosis, etc.) are not involved in mediating the drug's effect upon the aversive threshold. Pretreatment with 0.5 mg/kg of scopolamine methylbromide, which was by itself inactive in the spatial preference technique, eradicated all visible signs of peripheral parasympathomimetic stimulation (i.e., diarrhea and salivation) without blocking pilocarpine's ability to raise the aversive threshold. It, therefore, appears that pilocarpine is able to produce increments in the aversive threshold by exerting its activity primarily on the central nervous system. This conclusion is further supported by the fact that a central acting anticholinergic, scopolamine hydrobromide (1.0 mg/kg), was able to completely block the effects of pilocarpine (10.0 mg/kg) upon the aversive threshold (e.g. see Fig. 5). This evidence suggests that pilocarpine produces increments in the aversive threshold via its central cholinomimetic properties.

Since the ability of pilocarpine and eserine to raise the aversive threshold probably cannot be accounted for solely in terms of reduced motor activity or the general debilitating effects of peripheral cholinergic stimulation, it appears possible that these agents may elevate the aversive threshold by means of a direct analgesic effect on the central nervous system. This speculation is in agreement with previous reports in the literature which indicate that enhancement of cholinergic tone can produce significant analgesia. For example, several clinical studies [24] have noted that neostigmine, an anticholinesterase agent, was able to increase the threshold of pain in human subjects. More recent evidence in various animal species has corroborated this earlier clinical data. Oxotremorine, a central acting cholinomimetic agent, and eserine, an anticholinesterase, are active in the mouse tail-flick analgesic test [6,7]. Furthermore, the analgetic effects of eserine were not altered by pretreatment with 1.0 mg/kg of atropine methylnitrate [6], a peripheral acting anticholiner-

gic which blocked all signs of peripheral autonomic cholinergic stimulation. This evidence led these authors [6] to conclude that eserine exerted its analgetic effects by means of its activity on central rather than peripheral cholinergic sites. Furthermore, other reports [16] have indicated that a relationship exists between levels of brain acetylcholine and degree of analgesia produced by oxotremorine in the mouse tail-flick test. Analgesic potency was positively correlated with increased levels of brain acetylcholine [16]. Although these data are intriguing, a cautionary note should be sounded with regard to the relationship between enhanced cholinergic tone and analgesia. The above investigators [16] have pointed out that although a positive correlation between brain acetylcholine and analgesia did hold for oxotremorine, no such relationship held for a series of other cholinergic and analgesic agents in the mouse.

To summarize, the present data suggest that enhanced cholinergic tone via the administration of pilocarpine or eserine leads to increases in the aversive threshold of the rat to foot shock. These increments cannot be entirely accounted for by changes in motor activity or by the debilitating effects of enhanced peripheral cholinergic stimulation. It would appear that these elevations in the aversive threshold may have been the result of a direct analgetic response produced by these agents mediated through central cholinergic mechanisms.

The present data with regard to atropine sulfate and scopolamine methylbromide, along with an earlier report from this laboratory [15] concerning scopolamine hydrobromide, indicate that reductions in cholinergic tone via the administration of these anticholinergics do not produce reliable increments in the aversive threshold. As we have pointed out previously [10], however, the spatial preference technique is designed to measure increases in the aversive threshold. Only one shock intensity below the normal threshold (i.e., 30 $\mu$A) is presented during each testing session, while the other four intensities (60, 90, 120, 150 $\mu$A) are above threshold. Thus, decreases in the aversive threshold under drug conditions are virtually impossible to measure. Therefore, the present results cannot rule out the possibility that these anticholinergics may lower the aversive threshold. In this regard it should be noted that clinical evidence does exist which indicates that atropine and scopolamine have antianalgesic properties in human subjects [3]. Thus, it is possible that cholinergic stimulation via eserine or pilocarpine leads to an analgetic response in the rat, while decreased cholinergic tone could produce a reduction in the aversive threshold. The present data support the above speculation with regard to cholinergic stimulation, but provides no evidence with regard to the anticholinergics tested, indicating only that reduced cholinergic tone does not reliably augment the aversive threshold to foot shock in the rat.

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