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Modulation of the Aversive Qualities of Shock Through a Central Inhibitory Cholinergic System in the Rat[1]

VINCENT P. HOUSER[2]

Psychotropic Drug Laboratory, Veterans Administration Hospital, Perry Point MD 21902

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HOUSER, V. P. *Modulation of the aversive qualities of shock through a central inhibitory cholinergic system in the rat.* PHARMAC. BIOCHEM. BEHAV. 4(5) 561-568, 1976. – Evidence has been supplied which suggests that a central inhibitory cholingeric (i.e., muscarinic) system may be involved in modulating the aversive qualities of electric shock in the rat. Central cholinergic stimulation via the administration of pilocarpine or arecoline elevated the threshold for grid shock, while central acting anticholinergics (i.e., scopolamine and atropine) produced decrements in the threshold. Peripheral acting anticholinergics (e.g., methyl scopolamine, methyl atropine) were less potent than central acting drugs given in equivalent doses, while peripheral cholinergic stimulants (i.e., neostigmine, carbachol) were inactive. In addition, only the central acting stimulant pilocarpine, and not carbachol, was able to block the decrements noted in response to scopolamine hydrobromide administration. Finally, only arecoline, and not nicotine, was able to elevate the aversive threshold indicating that muscarinic receptor sites are probably involved in mediating the effects of central cholinergic stimulants.

Cholinergic mechanisms Aversive thresholds Electric shock Pilocarpine Arecoline Scopolamine

RECENT reports from this laboratory have indicated that cholinergic stimulation via the administration of either pilocarpine nitrate or eserine sulfate produces reliable dose-related elevations in the aversive threshold to electric shock in the rat [9,10]. Central acting anticholinergics, such as scopolamine hydrobromide and atropine sulfate, on the other hand, did not affect the threshold when given alone, but scopolamine was able to completely block the effects of pilocarpine on the threshold when it was given 20 minutes before pilocarpine administration [10]. Preliminary evidence has also been reported which suggests that pilocarpine does not elevate the aversive threshold indirectly through its peripheral parasympathomimetic effects. Scopolamine methylbromide was effective in blocking all the debilitating effects of enhanced peripheral cholinergic stimulation (i.e., diarrhea, salivation, miosis, etc.) without altering pilocarpine's effects on the aversive threshold [10].

Although the above data present suggestive evidence that central cholinergic systems may be involved in producing analgesia in the spatial preference technique, several questions remain unanswered. If stimulation of a central cholinergic system elevates the aversive threshold to electric shock, central cholinergic blockade might be expected to produce decrements in the threshold. Previous attempts to assay the effects of central acting anticholinergics [9,10] have utilized animals who are adapted to the spatial preference technique by repeated testing. Thus, the baseline control threshold values are relatively low (i.e., under 50 μA) making any reliable reductions in the threshold impossible to measure. The present report has attempted to explore the effects of central acting anticholinergics on animals which have had no previous exposure to the spatial preference technique and thus have high baseline thresholds. Drug-induced decrements in the threshold can thus be more easily detected.

A second question concerns the relative potencies of the central vs peripheral acting cholinergic drugs. If a centrally mediated cholinergic system is involved in modulating the aversive threshold, central acting anticholinergics should have greater activity than peripheral-acting agents. Conversely, central acting cholinergic stimulants should be more active than peripheral cholinomimetics. Thirdly, if anticholinergics do affect the aversive threshold, this effect should be blocked by central rather than peripheral acting cholinergic stimulants. Finally, some attempt should be made to determine whether muscarinic or nicotinic receptors are primarily responsible for the effects noted when

[1] All requests for reprints should be sent to the author at : RD No. 1, Box B95, Route 94, Chester NY 10918.

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cholinergic drugs are administered to animals subjected to the spatial preference technique. The present paper is an attempt to address these particular questions.

METHOD

Animals

Animals used in this experiment were 96 male Sprague-Dawley derived rats obtained from ARS/Sprague-Dawley, Madison, Wisconsin. They weighed 148–203 g at the beginning of each experiment.

Apparatus

The test chamber and apparatus have been described in detail elsewhere [8]. 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 μA). The shock stimulus was provided by a DC 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 [12]. 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, 6 days a week. An experimental session consists of five 10-min periods in which 5 separate current intensities (i.e., 30, 60, 90, 120, 150 μA) 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 5 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 criterion 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 μA), 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 μA was arbitrarily assigned. The number of crossing responses made during each shock intensity was also recorded for each animal.

The drugs administered in the present study consisted of scopolamine hydrobromide (0.05, 0.10, 0.25, 0.50, 1.0, 2.0 mg/kg), atropine sulfate (10, 20, 40 mg/kg), atropine methylnitrate (10, 20 mg/kg), scopolamine methylbromide (0.05, 0.10, 0.25, 0.50, 1.0 mg/kg), pilocarpine nitrate (5.0, 10.0, 20.0 mg/kg), carbachol (0.06, 0.125, 0.250, 0.350 mg/kg), neostigmine (0.025, 0.05, 0.10 mg/kg), arecoline (1.0, 2.0, 6.0, 12.0, 24.0, 48.0 mg/kg), and nicotine (0.25, 0.50, 1.0 mg/kg). All drugs were dissolved in 0.9% saline and administered intraperitoneally in a volume of 1.0 ml/kg. Unless otherwise stated, all drugs were given one-half hour before threshold testing.

The 96 animals were randomly divided into 16 six-animal drug groups. Fourteen of the 16 drug groups are presented in the figures contained in the Results section of this paper. Figures 1, 2, 3, 4, and 8 present 2 six-animal groups each for a total of 10 drug groups. The remaining Figures (i.e., 5, 6, 7, 9) present the results of 4 drug groups. The final 2 drug groups received either nicotine (0.25, 0.50, 1.0 mg/kg) or arecoline (1.0, 2.0, 6.0, 12.0 mg/kg) and these results are summarized verbally in the Results section. Some of the groups were subjected to the spatial preference technique for 10–15 sessions until relatively low threshold values were obtained before drugs were administered. Other groups were given drug immediately after 3 initial saline control sessions. The former procedure was used to measure elevations in the threshold while the latter was used to measure decrements. Each drug was given in several separate doses in the following weekly series. Saline was administered for the first 3 days of each weekly series followed by 3 days of a particular drug dosage. Animals were not tested on the seventh day of these weekly series.

RESULTS

Figure 1 presents the mean aversive threshold for 2 groups of rats subjected to various doses of the central acting anticholinergic, scopolamine hydrobromide. The first saline bar in Fig. 1 represents the first 3 days that animals were subjected to the spatial preference technique. Thus, baseline thresholds are relatively high. Scopolamine in all doses tested reliably reduced the threshold according to a two-factor within analysis of variance [11]. Dose-response relationships are difficult to ascertain from the data in Fig. 1 primarily because the baseline thresholds for the two groups were not equated. Within groups, however, the 1.0 mg/kg dose appeared to reduce the threshold to slightly lower levels than the 0.5 mg/kg dose. An analysis of the crossing data indicated that none of the doses of scopolamine reliably affected motor activity. Thus, the reductions in the threshold noted in Fig. 1 were not the result of changes in motor activity.

Figure 2 presents the mean aversive threshold for 2 groups of rats subjected to various doses of another central acting anticholinergic, atropine sulfate. As was the case with scopolamine in Fig. 1, all doses of atropine significantly reduced the aversive threshold below control

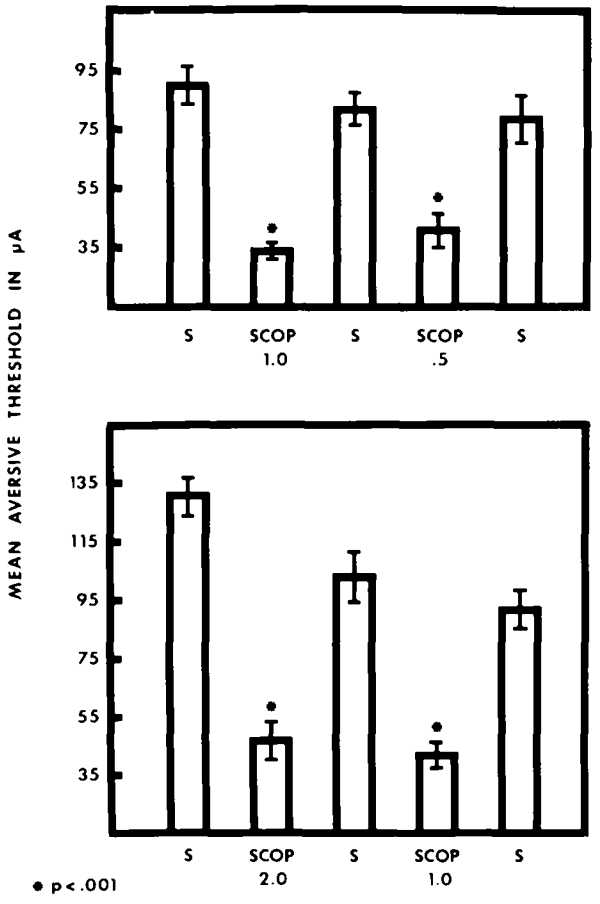

FIG. 1. Mean aversive threshold in μA with corresponding standard error of the means for 12 animals subjected to various doses of scopolamine hydrobromide. The top portion of the figure represents data from one 6-animal group, while the lower portion presents data from another 6 rats. Each bar represents the mean of 3 consecutive drug or saline (S) sessions. All dosages are given in mg/kg. Probability levels refer to comparisons (i.e., F tests) made between consecutive saline and drug sessions.

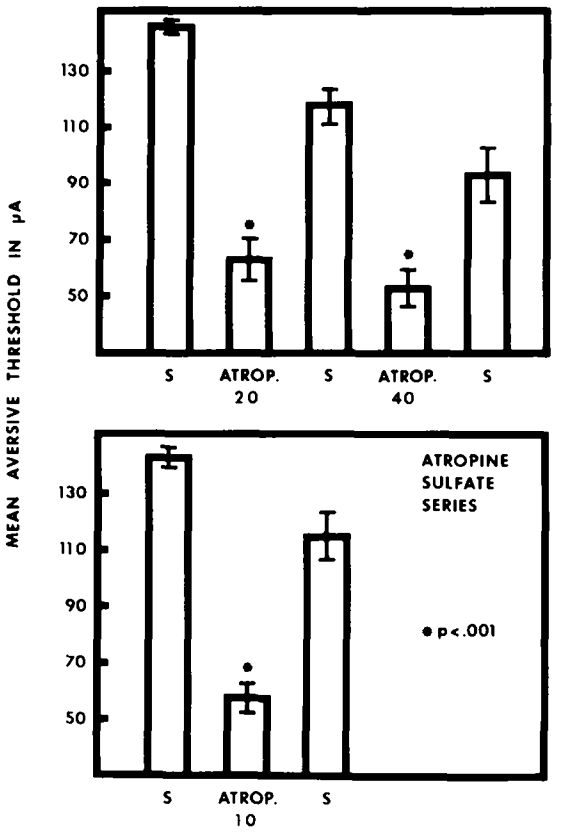

FIG. 2. Mean aversive threshold in μA with corresponding standard error of the means for 12 animals subjected to various doses of atropine sulfate. The top portion of the figure represents data from one 6-animal group, while the lower portion presents data from another 6 rats. Each bar represents the mean of 3 consecutive drug or saline (S) sessions. All dosages are given in mg/kg. Probability levels refer to comparisons (i.e., F tests) made between consecutive saline and drug sessions.

levels. Furthermore, the highest dose (i.e., 40 mg/kg) appeared to reduce the threshold to a slightly greater extent than the 20 mg/kg dose in one of the groups. Finally, an analysis of the crossing data indicated that this anticholinergic did not affect motor activity in any way. Thus, the changes in the threshold noted in Fig. 2 were not the result of an alteration in the number of escape responses emitted by the animals.

To explore whether the above two drugs produced decrements in the aversive threshold via their central or peripheral anticholinergic activity, the quaternary ammonium derivatives of these agents, atropine methylnitrate and scopolamine methylbromide, were administered to two groups of rats. Figure 3 presents the results of these manipulations by summarizing the mean aversive thresholds of animals subjected to these peripheral acting anticholinergics. As the data in this figure clearly indicate, these drugs were able to produce reliable decrements in the aversive threshold. These decrements, however, were not as severe as those seen in Fig. 1 and 2 when equivalent doses of the central acting agents were administered. For example, 1.0 mg/kg of scopolamine hydrobromide reduced the threshold by 58 μA while the same dose of scopolamine methylbromide reduced it by 42 μA. Atropine methylnitrate (20 mg/kg) was even less potent reducing the threshold by 23 μA, while an equivalent dose of atropine sulfate reduced the threshold by 80 μA. In addition, the initial presentation of both peripheral acting drugs significantly ($p<0.01$) reduced the number of crossing responses emitted by the animals, suggesting that the peripheral acting anticholinergics may have produced some debilitating effects that may have interfered with the execution of the escape response.

Although the data in Fig. 3 appear to indicate that equivalent doses of the peripheral acting anticholinergics are less potent than agents that have significant central activity, direct comparisons between various drug groups are difficult to make since baseline threshold values are not identical across groups. To control for this factor, two groups of animals were equated so that their initial baseline thresholds were identical. Then various doses of scopolamine hydrobromide and scopolamine methylbromide were administered to determine relative potencies. Figure 4 presents the results of these manipulations. As can be seen in this figure, the central acting anticholinergic was con-

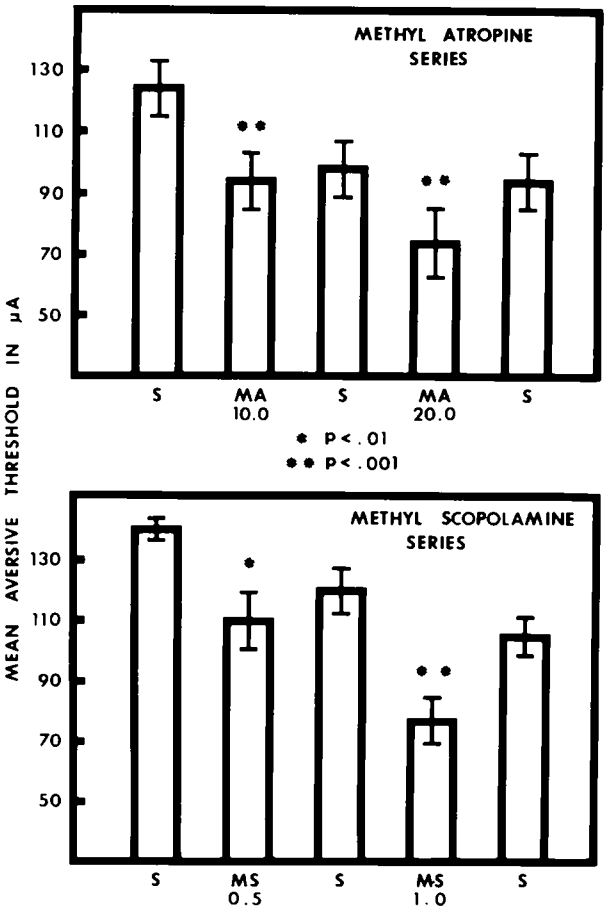

FIG. 3. Mean aversive threshold in μA with corresponding standard error of the means for 12 animals subjected to various doses of atropine methyl nitrate and scopolamine methylbromide. The top portion of the figure represents data from one 6-animal group, while the lower portion presents data from another 6 rats. Each bar represents the mean of 3 consecutive drug or saline (S) sessions. All dosages are given in mg/kg. Probability levels refer to comparisons (i.e., F tests) made between consecutive saline and drug sessions.

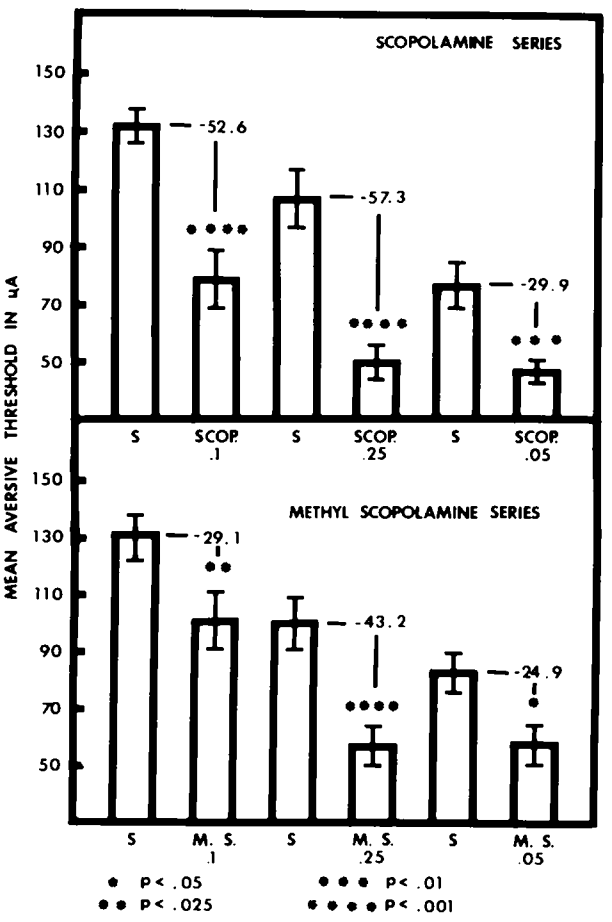

FIG. 4. Mean aversive threshold in μA with corresponding standard error of the means for 12 animals subjected to various doses of scopolamine hydrobromide (scopolamine) and scopolamine methylbromide (methyl scopolamine). The top portion of the figure represents data from one 6-animal group, while the lower portion represents data from another 6 rats. Each bar represents the mean of 3 consecutive drug or saline (S) sessions. All dosages are given in mg/kg. Probability levels refer to comparisons (i.e., F tests) made between consecutive saline and drug sessions. The negative numbers represent the differences generated by each dose level from the preceding saline sessions.

siderably more potent than scopolamine methylbromide in reducing the aversive threshold. This was especially the case when baseline thresholds were relatively high as in the 0.1 mg/kg dosage. The differences in drug potencies were reduced as the baseline (i.e., saline) thresholds fell making drug-induced reductions in the threshold more difficult to measure. It must be remembered that the spatial preference technique presents shock intensities which generate control thresholds that normally remain above 50 μA. Thus, a floor effect exists which precludes decrements below 50 μA. Finally, none of the drug dosages in Fig. 4 reliably affected the number of crossing responses emitted by the animals.

To summarize, the above data indicate that scopolamine hydrobromide is approximately 1.8 times as potent as scopolamine methylbromide. Even greater differences were found in the atropine data indicating that the central acting agent was approximately 3.3 times as potent as atropine methylnitrate. This, in turn, suggests that the reductions in the aversive threshold noted in response to the anticholinergics are probably a result of the activity of these agents at central cholinergic sites.

Once evidence was available concerning the affects of the anticholinergics upon the aversive threshold, attempts were made to explore, more fully, the effects of cholinomimetic agents using the spatial preference technique. Since earlier work had suggested that cholinergic stimulation had led to elevations in the threshold, animals were subjected to the spatial preference technique for at least 12 sessions or until relatively low (i.e., below 70 μA) baseline thresholds were obtained. Then various cholinomimetic agents were introduced to measure their effects on the aversive threshold.

Figure 5 presents the mean aversive thresholds and mean number of crossings made by animals subjected to various doses of pilocarpine nitrate. As the data in Fig. 5 indicate, pilocarpine was able to significantly elevate the aversive threshold in a dose-dependent manner. All doses of the drug that elevated the threshold, however, also reduced the number of crossing responses emitted by the animals.

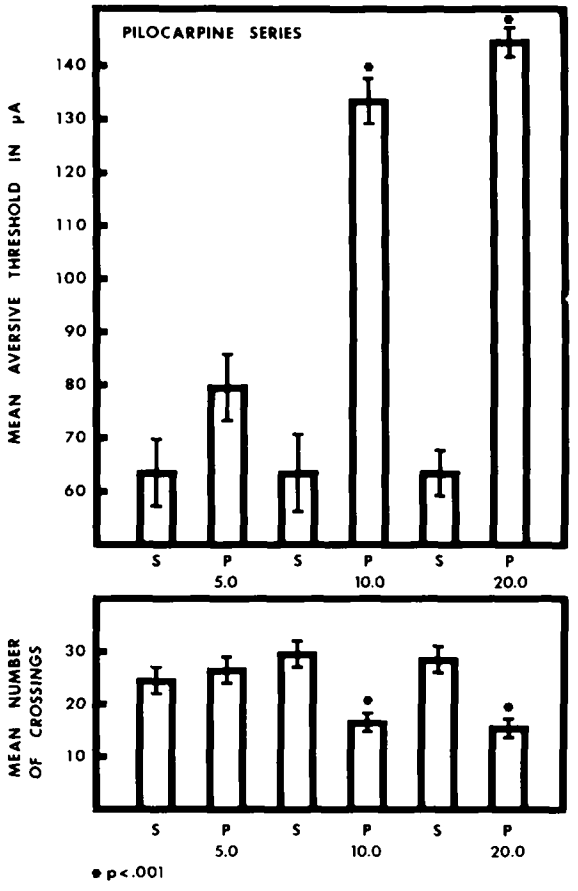

FIG. 5. Mean aversive threshold and mean number of crossings made by 6 animals subjected to various doses of pilocarpine nitrate. The first saline (S) bar represents the performance of animals after 15 sessions of pre-exposure to the spatial preference technique. Each bar represents the mean of 3 consecutive drug or saline (S) sessions. All dosages are given in mg/kg. Probability levels refer to comparisons (i.e., F tests) made between consecutive saline and drug dosage series.

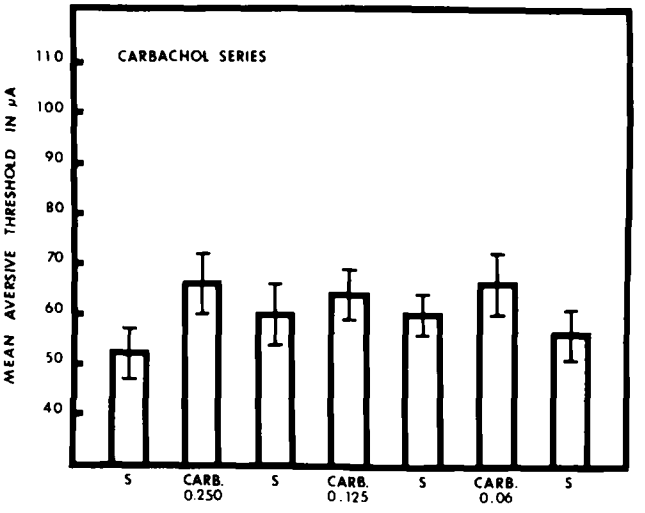

FIG. 6. Mean aversive threshold with corresponding standard error of the means for 6 animals subjected to various doses of carbachol. The first saline (S) bar represents the performance of animals after 15 sessions of pre-exposure to the spatial preferneee technique. Each bar represents the mean of 3 consecutive drug or saline (S) sessions. All dosages are given in mg/kg. Probability levels refer to comparisons (i.e., F tests) made between consecutive saline and drug dosage series.

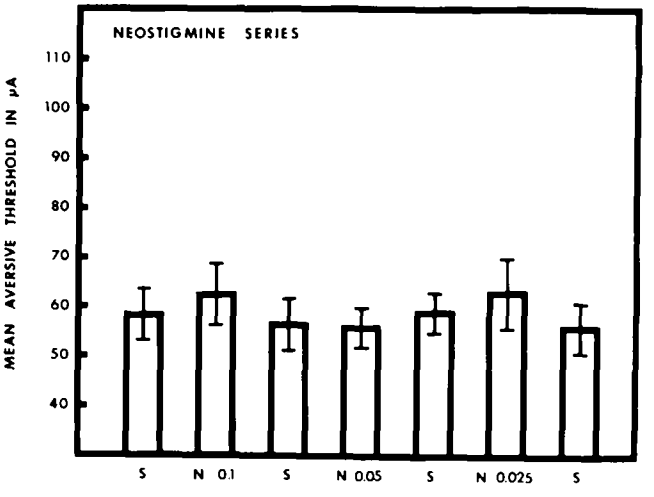

FIG. 7. Mean aversive threshold with corresponding standard error or the means for 6 animals subjected to various doses of neostigmine. The first saline (S) bar represents the performance of animals after 15 sessions of pre-exposure to the spatial preference technique. Each bar represents the mean of 3 consecutive drug or saline (S) sessions. All dosages are given in mg/kg. Probability levels refer to comparisons (i.e., F tests) made between consecutive saline and drug dosage series.

In order to determine whether the peripheral or central effects of pilocarpine were responsible for the above effects, several peripheral acting cholinergic stimulants were administered to animals who had relatively low baseline threshold values. Figure 6 presents the effects of administering various doses of carbachol, a peripheral acting cholinomimetic, to a group of rats subjected to the spatial preference technique. As the data in Fig. 6 clearly indicate, carbachol had no reliable effects on the threshold even though signs of severe parasympathetic stimulation were evident. Furthermore, none of the dosages in Fig. 6 significantly altered the number of crossing responses made.

Figure 7 presents the mean aversive thresholds for a group of rats who were administered various doses of neostigmine, a peripheral acting anticholinesterase. These results indicate that this drug did not affect the aversive threshold even though it was administered in relatively high doses that produced severe signs of peripheral cholinergic stimulation and in the highest two doses (i.e., 0.10 and 0.05 mg/kg) reliably ($p<0.025$) reduced the number of crossing responses emitted by the animals. Thus, this drug appeared to hamper the execution of the escape response through the debilitating effects of enhanced parasympathetic activity without significantly elevating the aversive threshold. The above data thus strongly suggest that pilocarpine probably produces its effects on the threshold via its central cholinergic properties.

Although the above results are suggestive of some type

of central involvement in the production of cholinergic drug effects on the aversive threshold, it is still possible that peripheral cholinergic blockade may contribute to the results observed in Fig. 1 and 2 since both methyl scopolamine and methyl atropine were able to significantly reduce the aversive threshold. To further explore this possibility two additional groups of animals were pretreated with either carbachol, a peripheral acting cholinomimetic, or pilocarpine nitrate, a central acting cholinergic stimulant, 20 min before the administration of scopolamine hydrobromide. If the peripheral actions of scopolamine hydrobromide are responsible for its effects on the threshold, both drugs should block its activity. If the central actions of scopolamine hydrobromide are responsible, however, only pilocarpine should block the reductions in the threshold noted after anticholinergic administration. And finally, if neither compound blocks scopolamine's effects it would suggest that decrements in the threshold are not mediated through the anticholinergic properties of scopolamine. Figure 8 presents the results of these pretreatment procedures upon the aversive threshold of two groups of rats who were equated for their initial baseline threshold values.

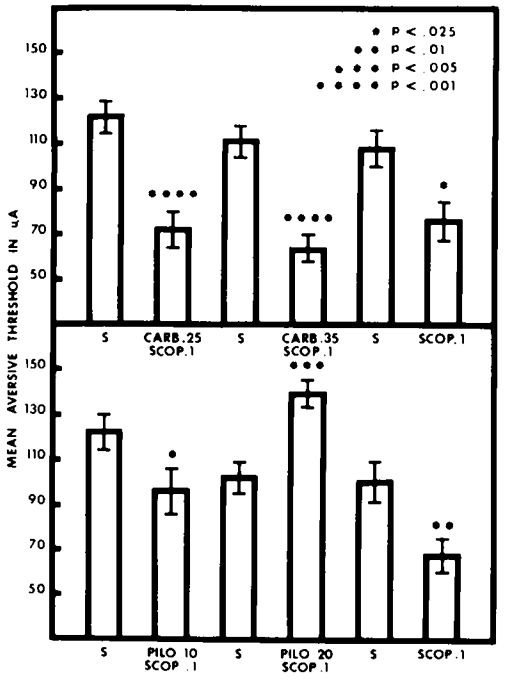

FIG. 8. Mean aversive threshold with corresponding standard error of the means for 12 rats pretreated with either carbachol (Carb.) or pilocarpine nitrate (Pilo.) 20 min before scopolamine (Scop.) hydrobromide administration. The top portion of the figure represents data from one 6-animal group, while the lower portion presents data from another 6 rats. Each bar represents the mean of 3 consecutive drug or saline (S) sessions. All dosages are given in mg/kg. Probability levels refer to comparisons (i.e., F tests) made between consecutive saline and drug sessions.

As these data clearly indicate, carbachol, even in doses as high as 0.35 mg/kg, was not able to block the decrements produced by scopolamine administration. Pilocarpine (20 mg/kg), on the other hand, was able to not only completely block scopolamine's effects, but actually significantly elevated the threshold above control values. In addition, an analysis of the crossing data indicated that none of the dosages in Fig. 8 affected the number of crossing responses made with the exception of the 20 mg/kg dose of pilocarpine which reliably ($p<0.01$) reduced motor activity. It could be argued that the differences in the ability of the above two drugs to block the effects of scopolamine were due to the fact that the dosages of the two agents were not equated. Pilocarpine was administered in larger doses than carbachol and thus should produce greater effects. In fact, however, carbachol is an extremely potent cholinomimetic which produces lethal effects at low doses. In the present case, 0.35 mg/kg of carbachol led to the death of one animal in the drug group pictured in Fig. 8 and thus another animal had to be added at a later date. This fact suggests that 0.35 mg/kg was the highest dosage that could be used without causing lethal effects. Dosages of this magnitude produce severe signs of peripheral cholinergic stimulation (i.e., diarrhea, salivation, etc.) Pilocarpine (20.0 mg/kg), on the other hand, although producing signs of enhanced parasympathomimetic activity, is not a dosage that even approaches the LD_{50} for this agent. Thus, although the dosages are not equivalent, the relative potencies of the two drugs more than compensate for this difference making the carbachol pretreatment, if anything, more likely to block scopolamine's peripheral effects than the dose of pilocarpine that was administered. The above evidence thus strongly suggests that scopolamine produces its effects on the threshold by means of its anticholinergic activity in the central nervous system.

Finally, since central cholinergic stimulation appears to be involved in elevating the aversive threshold to electric shock, it seemed reasonable to explore whether nicotinic or muscarinic mechanisms are involved. To this end, two groups of rats who had received previous exposure to the spatial preference technique were administered various doses of nicotine (0.25, 0.50, 1.0 mg/kg) or arecoline (1.0, 2.0, 6.0, 12.0 mg/kg). Neither drug significantly elevated the aversive threshold in any of the doses tested. Arecoline (12.0 mg/kg) did, however, reliably reduce the number of crossing responses made.

The above results were somewhat surprising, and thus a final experiment was carried out using even larger doses of arecoline. Higher doses of nicotine were not attempted since 1.0 mg/kg leads to violent seizures approximately 5 10 min after intraperitoneal injection. Figure 9 presents the results of the final arecoline study. As these data clearly indicate, arecoline reliably elevated the aversive threshold to electric shock in a dose-dependent manner in dosages at or above 24 mg/kg. In addition, all 3 doses in Fig. 9 significantly reduced the number of crossing responses emitted by the animals.

DISCUSSION

The above data appear to suggest that a central muscarinic inhibitory system may be involved in modulating the aversive threshold to electric shock in the rat. Thus, stimulation of this cholinergic system via pilocarpine, eserine [10], or arecoline may reduce the noxious qualities of electric shock, thus elevating the aversive threshold. Reductions in cholinergic (i.e., muscarinic) activity via atropine or scopolamine, on the other hand, remove the inhibitory influences of this system and thus enhance the aversive qualities of grid shock producing reductions in the threshold. Furthermore, evidence has been supplied which

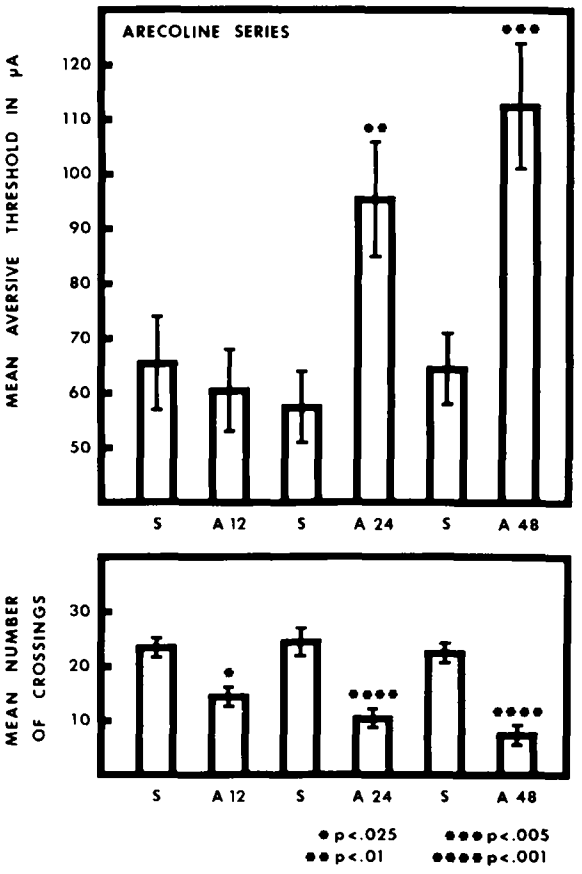

FIG. 9. Mean aversive threshold and mean number of crossings made by 6 animals subjected to various doses of arecoline. The first saline (S) bar represents the performance of animals after 15 sessions of pre-exposure to the spatial preference technique. Each bar represents the mean of 3 consecutive drug or saline (S) sessions. All dosages are given in mg/kg. Probability levels refer to comparisons (i.e., F tests) made between consecutive saline and drug dosage series.

suggests that this system is located in the central rather than the peripheral nervous system. For example, peripheral acting cholinergic stimulants (i.e., carbachol, neostigmine) are not able to elevate the threshold even when they are administered in doses that produce severe signs of peripheral parasympathomimetic activity and reduce the number of escape responses emitted. In addition, the effects of pilocarpine on the threshold can only be blocked by the central acting anticholinergic, scopolamine hydrobromide, and not the peripheral acting, scopolamine methylbromide [10]. Evidence is also available with regard to the anticholinergics which suggests that these drugs also produce their effects by exerting activity on the CNS. For example, peripheral acting anticholinergics are less potent than the more central acting agents, and only the central acting cholinergic stimulant, pilocarpine, was able to block the effects of scopolamine hydrobromide on the aversive threshold. Finally, evidence was presented which implicated muscarinic receptor sites in the mediation of the above effects. Arecoline and not nicotine was capable of elevating the aversive threshold. All the above data are consistent with the hypothesis that a central acting cholinergic (i.e., muscarinic) inhibitory system is involved in mediating the aversive qualities of electric shock in the rat.

This hypothesis, in turn, 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 [1,3] have noted that anticholinesterase agents were able to increase the threshold of pain in human subjects. More recent evidence in various animal species has corroborated these earlier clinical data. Oxotremorine, a central acting cholinomimetic agent, and eserine, an anticholinesterase, are active in the mouse tail-flick analgesic test [5,6]. In addition, the analgetic effects of eserine were not altered by pretreatment with 1.0 mg/kg of atropine methylnitrate [6].

Furthermore, both pilocarpine and eserine have been reported to exhibit significant analgesia in the rat using the radiant heat tail-flick assay [2]. In agreement with the present results, neostigmine was inactive in this test. Both scopolamine and atropine were able to block the analgesia produced by cholinergic stimulation in the tail-flick assay [2]. Similar findings have also been reported in response to eserine and pilocarpine administration in mice using the phenylbenzoquinone writhing test [7]. Thus, it would appear that central cholinergic stimulation can produce significant analgesia as measured by a variety of pharmacological tests.

One possible criticism of the present data might involve the fact that cholinergic stimulants were tested after animals had previous experience with the spatial preference technique and thus had low baseline thresholds, while the anticholinergics were tested during initial exposure to the procedure when thresholds were higher. This situation thus might have biased the results making elevations in the threshold more likely in the former case, and decrements more likely in the latter situation. In fact, however, we have reported the effects of both peripheral and central acting anticholinergics upon animals who had previous experience with the spatial preference technique and thus demonstrated low baseline threshold values. Both scopolamine hydrobromide and scopolamine methylbromide produced no elevations in the threshold [10]. Furthermore, recent unpublished work in our laboratory has indicated that pilocarpine given during the initial exposure to the technique does not produce decrements in the threshold. Thus, differences in baseline threshold do not account for the opposing effects of the cholinergic stimulants and the anticholinergics upon the aversive threshold.

As a final note, mention should be made of several possible alternative explanations that could account for the effects of cholinergic stimulants without recourse to the hypothesis that a central inhibitory cholinergic system may modulate the aversive threshold. Since most doses of the cholinergic stimulants (i.e., eserine, arecoline, pilocarpine) that elevate the threshold also produce decrements in the number of crossing responses emitted, it is possible that cholinergic stimulation of the CNS produces a direct sedative effect which interferes with the execution of the motor (i.e., escape) response, thus indirectly elevating the threshold. Several lines of evidence, however, argue against such an explanation. First, eserine was able to produce reliable increments in the aversive threshold at dosages (i.e., 0.5 mg/kg) that did not significantly reduce motor activity [10]. Secondly, neostigmine was able to produce significant decrements in motor activity without elevating the threshold. This fact indicates that a reduction in motor

activity does not always lead to increments in the threshold as computed in the spatial preference technique. Finally, if one were to hypothesize that cholinergic stimulation in the CNS leads to sedation, anticholinergics might be expected to produce hyperactivity. The crossing results with regard to the scopolamine hydrobromide and atropine sulfate data do not support this thesis. Thus, it is unlikely that drug-induced sedation can account for the elevations in the aversive threshold produced by central cholinergic stimulants.

Although gross changes in motor activity are unlikely to account for the alterations in threshold noted in response to cholinergic drugs, it is possible that the drugs might be modifying response style (e.g., defensive behaviors), thus making it seem that the threshold is altered. This possibility is difficult to rule out entirely, but to date, after visually observing many animals subjected to the spatial preference technique, we have noted no reliable alteration in the execution of the escape response under cholinergic drugs. More objective measures of response execution will have to be devised, however, before the above explanation can be rejected.

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