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The Effects of Scopolamine and Pilocarpine upon the Aversive Threshold of the Rat

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HOUSER, V. P. AND D. A. VAN HART. *The effects of scopolamine and pilocarpine upon the aversive threshold of the rat.* PHARMAC. BIOCHEM. BEHAV. 1(4) 427–431, 1973.–The analgesic potency of scopolamine hydrobromide (0.125, 0.250, 0.50, 1.0, 2.0 mg/kg) and pilocarpine nitrate (1.25, 2.50, 5.0, 10.0 mg/kg) were measured in the rat using the spatial preference technique. Only pilocarpine nitrate in doses at or above 2.50 mg/kg significantly raised the aversive threshold. These data were interpreted to indicate that cholinergic systems may be involved in the production of analgesia in the rat. The present results may be useful in interpreting the behavioral effects of cholinergic drugs administered to animals that are under the control of aversive schedules of reinforcement.

Scopolamine Pilocarpine Aversive thresholds Rats

PREVIOUS reports have indicated that drugs which modify cholinergic tone can influence behavior under the control of schedules of reinforcement that employ electric shock. 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, have differential effects upon behavior conditioned in the presence of electric shock, depending on many factors including the dose administered and species employed. Low doses of scopolamine (i.e., 0.1 mg/kg) have been reported to enhance the acquisition of Sidman avoidance behavior in rats by augmenting response rate and reducing shock rates [16,17]. On the other hand, doses above 0.4 mg/kg lead to a disruption of Sidman avoidance behavior in rats producing a consequent increase in shock rates [9]. The disruptive effect of scopolamine (0.06–1.0 mg/kg) upon a Sidman avoidance task has also been reported in squirrel monkeys [10,14]. Other types of behaviors acquired under schedules that employ electric shock are also affected by scopolamine. For example, conditioned suppression of drinking in response to a conditioned stimulus previously associated with shock was abolished in rats trained under scopolamine [1,2].

In attempting to account for the behavioral effects of scopolamine administered in aversive test procedures some investigators [16,17] have suggested that anticholinergics may alter the sensory characteristics of electric shock. This effect of scopolamine could occur by blocking perceptual adaptation or habituation to shock, thus increasing the apparent shock intensity. Scopolamine could also affect the sensory characteristics of electric shock by a peripheral mechanism which assumes that the reduced amount of sweating which occurs after the administration of anticholinergics increases tissue resistance, consequently altering the intensity of shock delivered to the animal. 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.

Previous attempts to measure the possible analgesic properties of cholinergic agents have proven difficult since the animal models typically used to measure analgesia do not always provide results that coincide with clinical data in man. For example, the hot plate test in which the experimenter measures latency of paw licking in response to a thermal stimulus indicates that cholinomimetic, as well as anticholinergic agents, are active analgesics [22] while these drugs are not clinically active in man [7]. To the contrary, at least one report [5] has indicated that anticholinergic agents (i.e., atropine and scopolamine) increase sensitivity to somatic pain in the doses generally used as preanesthetic medication in man.

The present report is an attempt to gain a more accurate assessment of the possible analgesic qualities of drugs which modify cholinergic tone using grid shock in the rat. Aversive thresholds were measured using the spatial preference technique [3] which allows animals to escape various shock intensities by simply crossing from one side of a cage to the other. Previous reports have indicated that this technique is sensitive to the narcotic [11], narcotic antagonist [12] and weak analgesics [13] without reacting to sedative doses of sodium pentobarbital [12]. Thus, the spatial preference technique is sensitive to relatively low doses of a wide variety of standard analgesic substances known to be clinically active in man. Furthermore, it has proven to be a reasonably selective test in that it does not detect analgesic potency in agents (i.e., sodium pentobarbital) that are clinically nonanalgesic in man, but which do possess strong central nervous system activity. Thus, the sensitivity and selectivity of this test makes it possible to reliably determine the analgesic properties of a wide variety of psychoactive com-

pounds. The present report provides information on the analgesic properties of two agents that modify cholinergic tone using the spatial preference technique. This type of data may be of value in interpreting some of the behavioral effects noted when these agents are administered to animals under the control of aversive schedules of reinforcement.

METHOD

Animals and Apparatus

Twelve male Sprague-Dawley rats, obtained from ARS/Sprague-Dawley, Madison, Wisconsin, were used in the present experiment. They weighed 200–243 g at the beginning of the experimental period. The test chamber and procedure have been described in detail elsewhere [11]. Briefly, the chamber consisted of a rectangular Plexiglas shuttle box 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 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 the session.

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 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 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 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 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 session was also recorded for each animal.

After ten sessions all animals demonstrated stable threshold values. Animals were then randomly assigned to two separate six animal drug groups. Each drug was given in several separate doses in an ascending 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.

The two drugs administered in the present study consisted of pilocarpine nitrate (1.25, 2.50, 5.0, 10.0 mg/kg) and scopolamine hydrobromide (0.125, 0.25, 0.50, 1.0, 2.0 mg/kg). Both drugs were dissolved in 0.9% saline and administered intraperitoneally (IP) in a volume of 1 ml/kg one-half hour before threshold testing.

RESULTS

Figure 1 presents the mean aversive thresholds with corresponding standard error of the means for the six animals who were administered pilocarpine nitrate in various dosages. As is clearly evident from the data in this figure, pilocarpine nitrate in doses at or above 2.5 mg/kg reliably raised the aversive threshold. A two-factor repeated measures analysis of variance [19] indicated that the three highest doses (2.5, 5.0, 10.0 mg/kg) produced increments in the aversive threshold that were statistically significant at probability levels in excess of .001. There did not appear to be a clear dose–response relationship reflected in the data since 2.5 mg/kg raised the mean aversive threshold to levels which approximated those under 10 mg/kg of the drug.

Previous reports from this laboratory [13] have described a method for computing ED_{50} values for agents that demonstrate activity in the spatial preference technique. Briefly, an arbitrary criterion has been established which allows one to determine if an animal has demonstrated an analgesic response to a particular drug dosage. This criterion is simply a 10 μA increase in the mean aversive threshold under a particular drug dose over and above the mean threshold computed for the preceding three saline days. If an animal meets this criterion, it is assumed it has demonstrated an analgesic response. This then allows one to compute what percentage of a six animal group demonstrates an analgesic response to a series of drug dosages. These percentage values can then be used to compute ED_{50} values according to the method of Litchfield and Wilcoxon [18]. These computations were made for pilocarpine nitrate which has an ED_{50} value of 2.00 mg/kg with 95% confidence intervals of 1.37–2.90 mg/kg.

Figure 2 presents the mean aversive thresholds with corresponding standard error of the means for those animals subjected to the various dosages of scopolamine hydrobromide. These data indicate that this anticholinergic agent was ineffective in altering the aversive threshold in any of the doses tested. A repeated measures analysis of variance [19] demonstrated that no significant differences occurred between drug and saline conditions.

Figure 3 presents the mean number of crossings made under saline and drug conditions for both the scopolamine and pilocarpine animals. Standard errors of the means are also included. As this figure indicates, scopolamine reliably

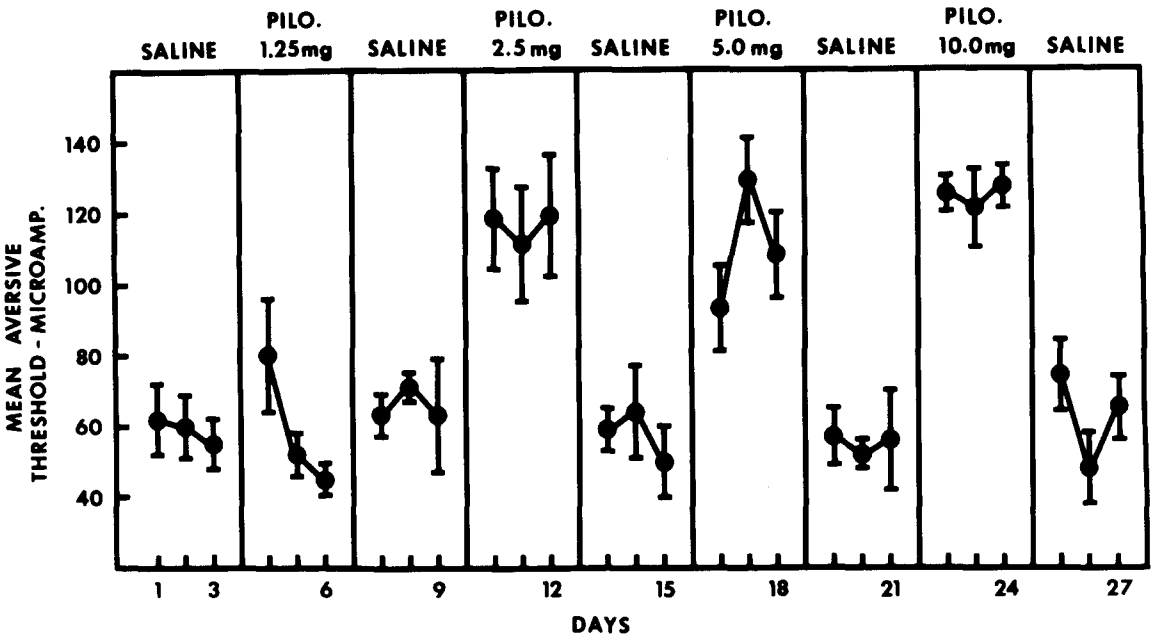

FIG. 1. Mean aversive threshold with corresponding standard error of the means for six animals under saline or various dosages (1.25, 2.5, 5.0, 10.0 mg/kg) of pilocarpine nitrate.

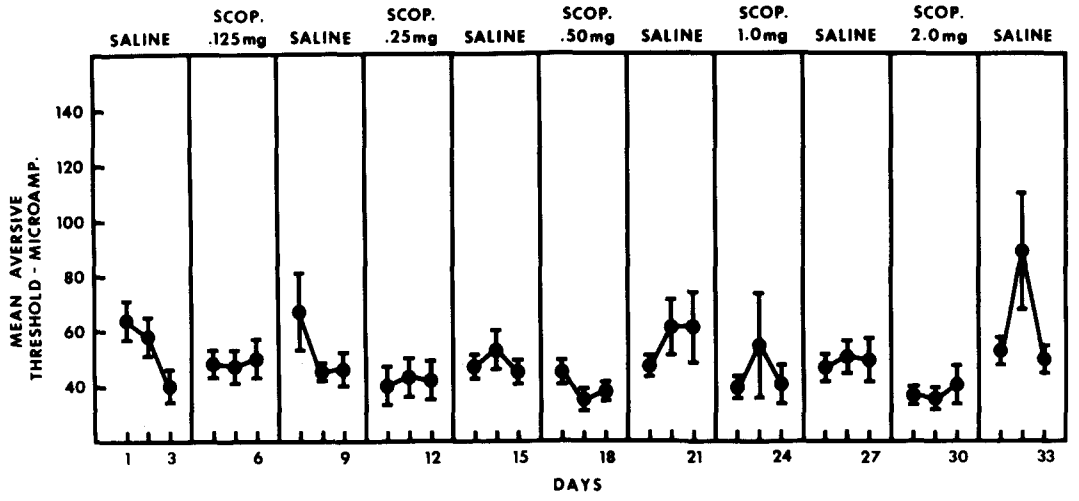

FIG. 2. Mean aversive threshold with corresponding standard error of the means for six animals under saline or various dosages (0.125, 0.25, 0.50, 1.0, 2.0 mg/kg) of scopolamine hydrobromide.

reduced the number of crossing responses made only under the highest dose (i.e., 2.0 mg/kg). This decrement in the number of crossings made under 2.0 mg/kg, although statistically significant, reflects the higher saline values at the end of the drug series rather than a reduction in this behavioral measure under drug conditions. Pilocarpine, on the other hand, significantly reduced the number of crossings made under the two highest dosages (5.0, 10.0 mg/kg). The pilocarpine data demonstrated a dose–response relationship in that higher doses produced greater decrements in this behavioral measure than did lower dosages.

DISCUSSION

The present results indicate that scopolamine hydrobromide does not lead to an analgesic response in the rat under a wide variety of dosages. These results are in agreement with the clinical literature [7] which indicates that anticholinergics do not have significant analgesic potency in man. Since the saline control aversive thresholds were near minimal levels (i.e., 45–55 μA), a reliable antianalgesic effect would be difficult, if not impossible, to detect. Thus, it is possible that scopolamine hydrobromide may produce

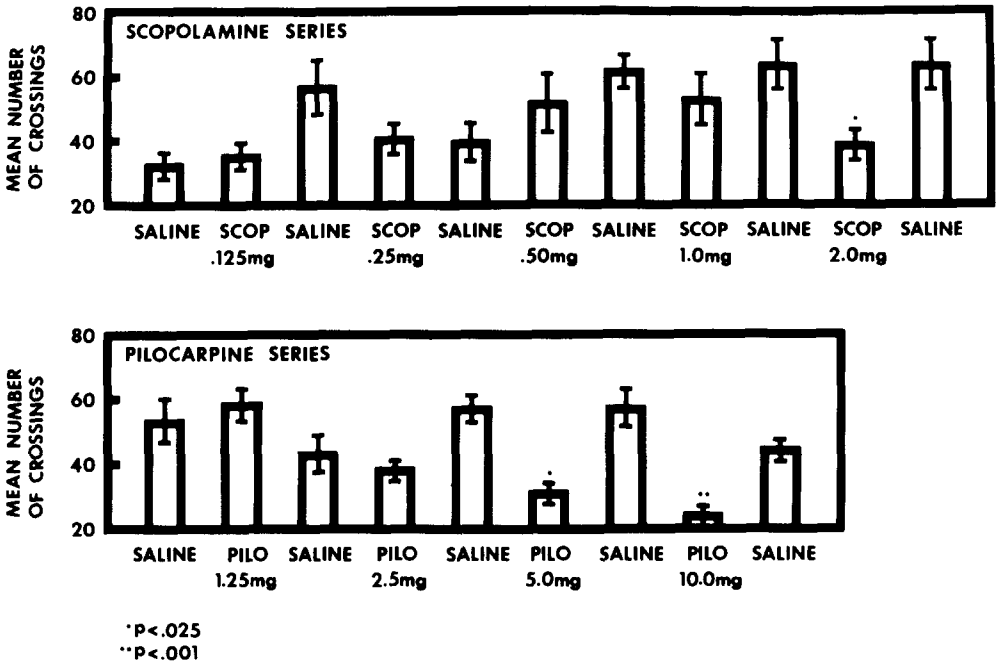

FIG. 3. Mean number of crossings with corresponding standard error of the means made by two (six animal) groups of rats under saline or various dosages of scopolamine hydrobromide (0.125, 0.25, 0.50, 1.0, 2.0 mg/kg) or pilocarpine nitrate (1.25, 2.5, 5.0, 10.0 mg/kg). Statistical probability levels were determined using a two-factor repeated measures analysis of variance [19].

antianalgesic effects in the rat which were not detected in this test. These data would then suggest that the performance decrements noted after scopolamine administration in rats subjected to aversive schedules of reinforcement [1,2] are probably not due to analgesia. The enhanced performance of Sidman avoidance behavior noted after low doses of the drug [9], however, could reflect the antianalgesic effects of the drug. The present results do not rule out such a possibility.

The results with regard to pilocarpine are more difficult to interpret. Cholinergic stimulants are not clinically known for their analgesic properties [7]. The animal literature, however, does suggest that cholinergic stimulation may lead to analgesia. For example, agents which act as cholinergic stimulants (i.e., physostigmine, oxotremorine) are active in the mouse tail-flick test [8] and hot plate test [4], while tremorine dihydrochloride, a cholinomimetic agent, is also active in the hot plate test [22]. Furthermore, cholinomimetic drugs (i.e., physostigmine, neostigmine and pilocarpine) are known to potentiate the effects of the nonnarcotic analgesics [15], as well as the narcotic antagonist analgesics [8]. Thus, it would appear, from both the previous work in mice cited above and the present data in rats, that cholinergic stimulants may have analgesic properties in these two species.

The results with regard to pilocarpine's effects on locomotor activity are in agreement with the previous literature. For example, previous reports [6] have noted that pilocarpine (2.5–10.0 mg/kg) produces a period of behavioral inhibition which lasts approximately one hour followed by a longer period of behavioral excitation. Since the period of behavioral inhibition occurred during the majority of the testing procedure, it was not surprising the number of crossings measure demonstrated a reliable dose-related decrement (see Fig. 3).

Since pilocarpine was able to reliably reduce the number of crossings made under the higher dosages, it could be argued that the increments in the aversive threshold merely reflected the sedative properties of the drug. Thus, cholinergic stimulation may lead to inhibition of movement (i.e., sedation) which, in turn, leads to higher threshold values, without directly affecting pain sensitivity. This explanation does not account, however, for the fact that 2.5 mg/kg of pilocarpine reliably augmented the aversive threshold without producing decrements in the number of crossings made. Thus, under 2.5 mg/kg the animals were making the same number of crossing responses, but their latency of response to shock onset was considerably increased, leading to higher thresholds. This fact clearly suggests that the increments in the aversive threshold produced by pilocarpine were probably not due to the drug's sedative properties, since the aversive threshold was augmented in doses (i.e., 2.5 mg/kg) that did not affect the number of crossing responses made.

To summarize, the present results indicate that the cholinomimetic agent, pilocarpine nitrate, demonstrated analgesic properties in the rat, whereas scopolamine hydrobromide given in a wide variety of doses did not. This information may be useful in evaluating the behavioral effects of cholinomimetic agents administered to rats whose behavior is controlled by aversive schedules of reinforcement that utilize electric shock. Thus, the decrements in conditioned avoidance behavior noted after pilocarpine administration [20] may be due, in part, to the drug's analgesic properties.

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