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Changes in the Aversive Threshold of the Rat Produced by Adrenergic Drugs

VINCENT P. HOUSER AND DALE A. VAN HART

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

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HOUSER, V. P. AND D. A. VAN HART. *Changes in the aversive threshold of the rat produced by adrenergic drugs.* PHARMAC. BIOCHEM. BEHAV. 1(6) 673 678, 1973.–An attempt was made to assay the analgesic potency of d-amphetamine sulfate (0.5, 1.0, 2.0, 4.0 mg/kg) and α-methyl-p-tyrosine (75, 150, 225 mg/kg) in the rat using the spatial preference technique. Amphetamine in all doses tested significantly raised the aversive threshold while α-methyl-p-tyrosine demonstrated similar effects only with the 150 and 255 mg/kg dosages. These data were interpreted to suggest that α-MT raised the aversive threshold by mechanisms other than drug-induced sedation, while amphetamine produced similar results by a direct analgesic effect and/or by altering locomotor activity. It was suggested that intact adrenergic systems may be needed for animals to fully respond to the aversive qualities of electric shock.

D-amphetamine sulfate α-methyl-p-tyrosine Aversive thresholds Rats

PREVIOUS reports have indicated that manipulation of adrenergic tone can lead to changes in pain sensitivity in various species. For example, the latency to a licking response in the mouse subjected to the hot-plate test is increased after the administration of several doses of methamphetamine [23]. Furthermore, the aversive threshold in the monkey as determined through the use of a titration schedule has been reported to be elevated in response to either d-amphetamine sulfate or methamphetamine administration [14,25]. Likewise, reduced adrenergic tone produced by the administration of α-methyl-p-tyrosine (α-MT) has been reported to substantially raise the aversive threshold to tail shock in squirrel monkeys subjected to a titration schedule [14]. Additional indirect evidence also indicates that adrenergic systems can influence the degree of morphine analgesia in various species. Pretreatment with reserpine has been reported to reduce morphine's analgesic potency when tested in mice using the tail-flick procedure [7], or the hot-plate test [24]. In addition, reserpine was effective in blocking morphine analgesia in the rabbit using electrical stimulation of the tooth pulp [24]. Pretreatment with α-MT was also able to block morphine's analgesic activity in the hot-plate test [24]. Amphetamine, on the other hand, administered to mice prior to morphine was effective in enhancing the analgesic properties of morphine tested in the mouse tail-flick test [7]. Similar effects have been noted when amphetamine was administered prior to codeine in the rat tail-clip test [1].

The previous work cited above, however, has assayed the potency of various drugs which alter adrenergic tone using analgesic tests which do not detect analgesia for all classes of agents known to be clinically active in man [11,12]. Thus, animal models such as the tail-flick procedure may fail to detect analgesia in agents known to be effective in man [7], while other procedures such as writhing induced by chemicals may react to a host of agents which are clinically nonanalgesic [22].

The present paper summarizes the effects of drugs which modify adrenergic tone upon the aversive thresholds as measured by the spatial preference technique in rats. This technique was first introduced by Campbell [3,4] with regard to determining detection, aversion, tetanization, and death thresholds. Upon subsequent investigation other laboratories used the spatial preference technique to explore the role of age, sex, and strain variables upon the aversive threshold of the rat [18] and to determine the analgesic potency of such drugs as aspirin and meprobamate [2]. Our laboratory has modified Campbell's procedure so that repeated measures may be taken on individual animals. Previous reports from our laboratory have indicated that this modified 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 narcotic analgesics (i.e., morphine [10], codeine, meperidine hydrochloride [11]), weak analgesics (i.e., sodium salicylate, indomethacin [12]) as well as the narcotic antagonist analgesics (i.e., pentazocine, cyclazocine [11]). The procedure also appears to be somewhat selective in that sedative doses of sodium pentobarbital which have been reported to be nonanalgesic in man [9] are also inactive in the spatial preference technique [11]. Finally, a recent report from this laboratory [13] has indicated that another clinically nonanalgesic agent, scopolamine hydrobromide, in a wide range of doses (i.e., .125 - 2.0 mg/kg) did not affect the aversive threshold as measured by the spatial preference technique, even though this anticholinergic has been reported to affect a wide variety of other behaviors

[6]. Thus, although this procedure will require further replication, especially by other laboratories, the preliminary results indicate that the spatial preference technique may be a more sensitive measure of drug-induced analgesia than previous analgesic assays. Thus, the present report by utilizing this technique could supply more definitive information as to whether drugs which alter adrenergic tone are, by themselves, able to affect the aversive threshold in rats.

METHOD

Animals and Apparatus

Twelve male Sprague-Dawley derived rats obtained from Charles River Laboratories, North Wilmington, Massachusetts, were used in the present study. They weighed 130–161 gms at the beginning of the experiment. The test chamber and procedure have been described in detail elsewhere [10]. 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 [20]. 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 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 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 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.

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 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 two drugs administered in the present study consisted of d-amphetamine sulfate (0.5, 1.0, 2.0, 4.0 mg/kg) and the methyl ester hydrochloride of dl α-methyl-p-tyrosine (75, 150, 225 mg/kg). Both drugs were dissolved in 0.9% saline and administered intraperitoneally (IP) in a volume of 1 ml/kg. D-amphetamine was administered one-half hour before threshold testing. Since α-MT has been shown to produce its peak physiological effects (i.e., depletion of endogenous catecholamines) several hours after administration [19] and because single large doses are more toxic than multiple small doses [19], this drug was administered in 3 injections several hours before testing. The 75 mg/kg dosage was administered in three 25 mg/kg injections 4, 3 and 2 hr before testing. The 150 mg/kg dosage was given in three 50 mg/kg injections 4, 3, 2 hr and 8, 7, 6 hr before testing. Finally, the 225 mg/kg dosage was administered in three 75 mg/kg injections 8, 7 and 6 hr before testing.

RESULTS

Figure 1 presents the mean aversive thresholds and standard errors of the means for the two groups of animals subjected to either amphetamine (0.5, 1.0, 2.0, 4.0 mg/kg) or α-MT (75, 150, 225 mg/kg). This figure indicates that amphetamine was able to raise the aversive threshold in a dose related manner with the two higher doses (2.0, 4.0 mg/kg) raising the aversive threshold to near maximal levels while the 1.0 mg/kg dosage elevated it to a lesser degree. The 0.5 mg/kg dosage elevated the mean threshold to approximately the same level (i.e., 78 and 85 μA) both times it was administered. A two factor (within) analysis of variance [17] was performed on the threshold data and the results are summarized in Fig. 1. Amphetamine was able to raise the aversive threshold for all doses at or above 1.0 mg/kg to levels that were statistically significant ($p<0.01$). Although the 0.5 mg/kg dose raised the aversive threshold to nearly equivalent levels both times it was administered, the saline control sessions preceding the second 0.5 mg/kg series were somewhat lower than the saline sessions that preceded the first administration of this dosage. Therefore, since the statistical comparisons were made between consecutive saline and drug sessions, the second 0.5 mg/kg series proved to be significantly above ($p<0.05$) saline values while the first 0.5 mg/kg series was not. These data suggest that amphetamine in doses above 0.5 mg/kg can reliably elevate the aversive threshold of rats to electric shock.

Previous reports from this laboratory [12] have described a method for computing ED_{50} values for agents that de-

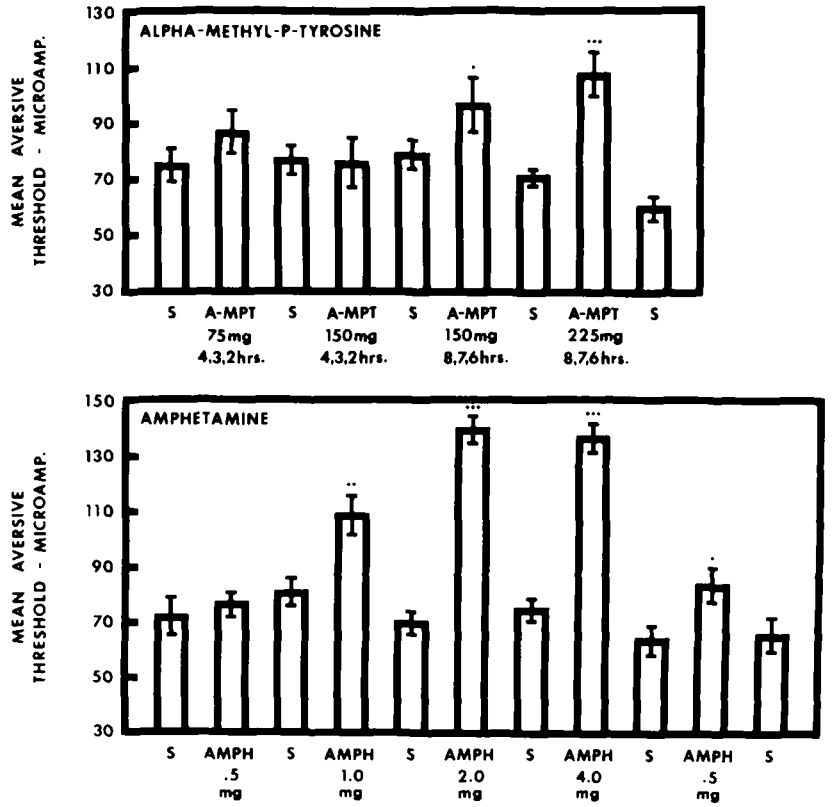

FIG. 1. Mean aversive thresholds and standard error of the means for those animals subjected to amphetamine (0.5, 1.0, 2.0, 4.0 mg/kg) or α-MT (75, 150, 225 mg/kg). Each bar represents the mean of 6 animals during three consecutive saline (S) or drug sessions. The amount of time between the three individual injections of α-MT and testing are listed in the figure. Probability levels for the various comparisons are listed as follows: $*p<0.05$, $**p<0.01$, $***p<0.001$.

monstrate 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 the animal meets this criterion, it is assumed that the rat 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 [16]. These computations were made for amphetamine which has an ED_{50} value of 0.50 mg/kg with 95% confidence intervals of 0.48–0.52 mg/kg.

The α-MT data contained in Fig. 1 indicate that this drug was able to significantly raise the aversive threshold in doses at or above 150 mg/kg when administered 8, 7 and 6 hr before testing according to a two factor (within) analysis of variance [17]. The drug was not able to raise the aversive threshold significantly when it was administered 4, 3, and 2 hr before testing in either the 75 mg/kg or 150 mg/kg dosage.

Figure 2 presents the mean number of crossing responses with corresponding standard error of the means for those animals subjected to either amphetamine or α-MT. Each bar represents the mean number of crossing responses made by the 6 animal group during three consecutive drug or saline sessions computed by averaging the total number of responses made during each session under all five shock intensities. These data indicate that amphetamine was able to significantly raise the total number of crossing responses made during the drug sessions only under the middle range (i.e., 1.0, 2.0 mg/kg) of doses. The lowest (i.e, 0.5 mg/kg) and highest (i.e., 4.0 mg/kg) doses showed no significant changes from preceding saline values. Alpha-MT was even less potent in this regard in that only the highest dose (225 mg/kg) was able to produce significant decrements in this behavioral measure. Thus, to summarize the session crossing data, amphetamine was able to significantly augment the number of responses made only through the middle ranges of doses while α-MT produced decrements in this response only under the highest dose.

The crossing data were also analyzed to determine if the individual drug dosages affected locomotor activity differentially during the various shock intensities presented within sessions. According to a two-factor (within) analysis of variance [17] amphetamine (1.0, 2.0 mg/kg) was able to significantly raise ($p<0.01$) the number of crossing responses made during the four highest intensities (i.e., 60, 90, 120, 150 μA), while not affecting this response during the 30 μA shock intensity. The first time the lowest (i.e., 0.5 mg/kg) dosage was administered it did not affect the num-

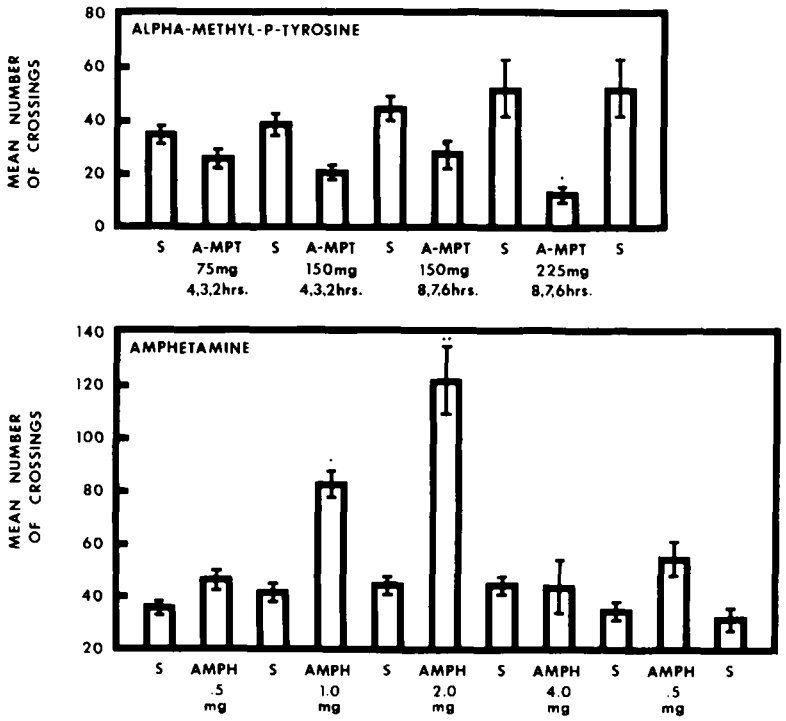

FIG. 2. Mean number of crossing responses and standard error of the means for those animals subjected to amphetamine (0.5, 1.0, 2.0, 4.0 mg/kg) or α-MT (75, 150, 225 mg/kg). Each bar represents the mean number of crossings made by 6 animals during three consecutive saline (S) or drug sessions. The amount of time between the three individual injections of α-MT and testing are listed in the figure. Probability levels for the various comparisons are listed as follows: $*p<0.005$, $**p<0.001$.

ber of crossings made at any shock intensity, but the second time this dosage was administered it significantly ($p<0.05$) augmented this measure only during the 60 and 90 μA shock intensities. Finally, the 4.0 mg/kg dosage significantly ($p<0.001$) decreased the number of crossing responses made during the 30 μA intensity, while augmenting this measure during the two highest intensities (i.e., 120, 150 μA). The 4.0 mg/kg dosage had no effect on locomotor activity during the 60 and 90 μA intensities.

The analysis of variance performed on the α-MT crossing data indicated that all the dosages significantly ($p<0.025$) reduced the number of crossings made during the lowest (i.e., 30 μA) shock intensity. Locomotor activity during all the higher intensities was unaffected by the drug, with the exception of the 225 mg/kg dosage which significantly ($p<0.05$) reduced the number of crossings made during the 90 and 150 μA shock intensities.

DISCUSSION

The present results indicate that both amphetamine and α-MT can reliably raise the aversive threshold to foot shock in the rat. D-amphetamine sulfate can produce this effect at doses as low as 0.5 mg/kg. Alpha-MT elevates the aversive threshold at doses at or above 150 mg/kg when administered in three equivalent injections 8, 7 and 6 hr before testing. The fact that amphetamine raised the aversive threshold is in agreement with other reports which indicate that amphetamine [14] and methamphetamine [25] were able to elevate the level of current delivered to monkeys who were performing under a titration schedule. Furthermore, amphetamine and other sympathomimetic amines are known to raise the pain threshold in dogs [9]. The present evidence could be interpreted to indicate that the rat may now be included in the various species that demonstrate an analgesic response to amphetamine.

Other explanations, however, which take into account the fact that amphetamine affects locomotor activity could be used to account for the present results. D-amphetamine sulfate is a known locomotor stimulant [21]. Thus, it is possible that the increase in the aversive threshold noted after the administration of all doses of d-amphetamine represented a reduction in passive avoidance behavior normally exhibited by animals under saline conditions. The locomotor stimulating effects of the drug at these dosages may have caused the rats to exhibit less freezing behavior in the nonshock portion of the cage, thus leading animals to cross back to the shock side more frequently than under saline conditions. Earlier work [5] has indicated that 0.5 mg/kg of amphetamine improved the acquisition of an active avoidance task in a shuttle box, while at the same time impairing passive avoidance behavior in the same apparatus. It has also been reported [15] that d-amphetamine in several doses (i.e., 0.5, 2.0, 5.0 mg/kg) reduced the amount of freezing behavior in both avoidance and nonavoidance test conditions. Furthermore, under avoidance conditions animals administered all three doses of the drug

demonstrated less freezing behavior and acquired an active avoidance response more rapidly in a shuttle box than control animals [15].

The present data are consistent with the above explanation in that all dosages of amphetamine that reliably augmented the aversive threshold also increased the number of crossing responses made during the higher shock intensities. Furthermore, the degree of elevation in the aversive threshold was reflected in the number of crossings data. Thus, when no elevation in the threshold was noted (i.e., during the first administration of 0.5 mg/kg of amphetamine) there were no changes in locomotor activity noted during the presentation of any of the shock intensities. When a moderate elevation in the aversive threshold was recorded (i.e., during the second administration of 0.5 mg/kg) locomotor activity was augmented only during the 60 and 90 μA shock intensities. Finally, when maximum increases in the aversive threshold were noted (i.e., 1.0, 2.0, 4.0 mg/kg) locomotor activity was augmented during the two highest shock intensities. This correlation between locomotor activity and the aversive threshold strongly suggests that the increase in the aversive threshold may have been a function of the locomotor stimulating effects of amphetamine. This explanation does not exclude the possibility, however, that amphetamine may also raise the aversive threshold to foot shock in the rat by means of a direct analgesic effect.

The fact that α-MT also raised the aversive threshold is interesting since it indicates that reduction in adrenergic tone via the depletion of endogenous catecholamines leads to an increase in the aversive threshold as does adrenergic stimulation (i.e., administration of amphetamine). The present data with regard to α-MT support previous reports [14] which indicated that this drug produced an analgesic response to tail shock in the squirrel monkey using similar dosing schedules (i.e., 150, 225 mg/kg, 8, 6, and 4 hr before testing). Furthermore, these data indicate that peak analgesic activity occurs approximately 8 hr after initiation of the injection schedule. The 150 mg/kg dosage was ineffective in raising the aversive threshold when animals were tested 4 hr after injection, but reliable increments were noted if they were tested 8 hr after the initiation of the dosing schedule. Previous reports [19] have noted that multiple injections (i.e., 3 × 50 mg/kg administered every 4 hr) of α-MT produce maximum depletion of rat brain norepinephrine and dopamine stores 12 hr after initiation of the dosing schedule. These data suggest that even greater effects might have been noted in the present report if a greater time span (i.e., 12 hr) had elapsed between drug administration and testing. Nevertheless, the fact that greater analgesia occurred when animals were tested during periods which should correspond to the time intervals of the greatest depletion of endogenous brain catecholamines may suggest that intact adrenergic systems are needed for animals to fully respond to the aversive qualities of electric shock.

The reduction in the mean number of crossing responses made during the lowest shock intensity (i.e., 30 μA) under all dosages of α-MT is in agreement with previous reports [8,21] which demonstrated that α-MT depressed locomotor activity. The fact that the aversive threshold was augmented in doses (i.e., 150 mg/kg) that did not significantly affect the number of crossings made during the four highest shock intensities, however, suggests that reduction in locomotor activity (i.e., sedation) cannot entirely account for the effects of the drug upon the aversive threshold. The apparent decrease in mean locomotor activity noted in Fig. 2 for the three lower doses, simply reflects a reduction in the relatively high number of crossing responses normally made during the 30 μA shock intensity. Under saline conditions animals make many crossing responses during this period since this intensity is subthreshold, and thus not aversive. Normally fewer crossing responses are made at the higher intensities since animals tend to remain on the nonshock portion of the tilt cage for the majority of the time these intensities are presented. The present data clearly indicate that animals given 150 mg/kg of α-MT demonstrated no reduction in the number of crossings made during the presentation of the four higher intensities (i.e., 60, 90, 120, 150 μA) even though their escape latencies were augmented, thus significantly elevating the aversive threshold. This suggests that a reduction in locomotor activity cannot be used to account for the increase in the aversive threshold under this dosage of the drug. Since locomotor activity was significantly reduced during the 90 and 150 μA shock intensities under 225 mg/kg of α-MT, it is possible that the elevations in the aversive threshold at this dosage did reflect the sedative or toxic effects of the drug.

In summary, it would appear that α-MT was able to raise the aversive threshold by mechanisms other than drug-induced sedation, while amphetamine produced similar effects by a direct analgesic effect and/or by altering locomotor activity.

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