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Serotonin and the aversive threshold in rats*

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The present study explored the effects of p-chlorophenylalaline (p-CPA—300, 200, 100, 50 mg/kg) and 5-hydroxytryptophan (5-HTP—15, 40, 60, 70, 95, 125, 175 mg/kg) upon the aversive threshold of the rat using the spatial preference technique. p-CPA had no reliable effects on the aversive threshold or upon motor activity as measured by this technique. 5-HTP, on the other hand, significantly raised the aversive threshold to moderate levels under the 95- and 125-mg/kg dosages, while motor activity was reduced under the 60-, 70-, 95- and 125-mg/kg dosages. p-CPA (200 mg/kg) was able to block both the increased thresholds and reduced motor activity noted after 125 mg/kg of 5-HTP. These results were interpreted to suggest that modulation of serotonergic activity has little effect upon the aversive threshold of the rat.

Serotonin has been implicated as one brain amine that may play a significant role in altering pain sensitivity in a number of animal species. For example, Tenen (1967) has reported that the administration of p-chlorophenylalanine, a serotonin depleting agent, to rats leads to a reduction in the pain threshold as measured by the flinch-jump method. Other evidence (Lee & Fennessy, 1970) has suggested that there is a correlation between morphine-induced analgesia and reduced levels of serotonin in the mouse brain Furthermore, it has been reported that p-chlorophenylalanine (p-CPA) antagonizes the analgesic effects of morphine (Fennessy & Lee, 1970; Tenen, 1968). These findings have led Tenen (1968) to suggest that morphine analgesia is mediated by the release of serotonin.

All the above data is consistent with the hypothesis that reductions in serotonergic activity, via the administration of p-CPA, leads to a reduction in the pain threshold, while elevations in serotonergic activity may produce analgesia. Although this simplistic model is attractive, other evidence suggests that serotonergic systems may have contrasting effects upon behavior acquired in the presence of electric shock. Stein, Wise, and Berger (1973) have presented data which suggest that a serotonergic system may mediate the behaviorally suppressive effects of punishment (i.e., usually electric shock). Support for this concept is found in a report by Geller and Blum (1970) who note that p-CPA attenuated the suppression of barpressing behavior normally noted when each response was paired with a brief electric shock. These results are difficult to interpret if one assumes that p-CPA lowers the pain threshold for electric shock.

The above contrasting evidence suggests that the effects of manipulating serotonergic tone upon pain sensitivity are far from clear. The present report is an attempt to explore the effects of p-CPA and 5-hydroxytryptophan, a precursor of serotonin, upon the aversive threshold of rats using the spatial preference technique. This technique has proven to be an extremely sensitive and reliable measure of drug-induced analgesia reacting to a host of agents known to be clinically active in man [e.g., morphine (Houser & Paré, 1972), pentazocine, cyclazocine (Houser & Paré, 1973a), and sodium salicylate (Houser & Paré, 1973b)]. Thus, by utilizing this technique, the present report may supply more definitive evidence as to whether serotonin plays a role in mediating the aversive qualities of electric shock.

METHOD

Subjects

Twelve male Sprague-Dawley derived rats obtained from ARS/Sprague-Dawley, Madison, Wisconsin, were used in the present study. They weighed 286-321 g at the beginning of the experiment.

Apparatus

The test chamber and apparatus have been described in detail elsewhere (Houser & Paré, 1972). 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 leverswitch 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. The shock stimulus was provided by a dc generator which produced a 60-Hz square wave output (Reus, Houser, & Paré, 1971).

Procedure

Each animal was subjected to a 50-min experimental session, the same time each day, 6 days a week. An experimental session consisted of five 10-min periods in which five separate current intensities (i.e., 30, 60, 90, 120, 150, microA) were presented in an ascending order. The shock was presented continuously 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.

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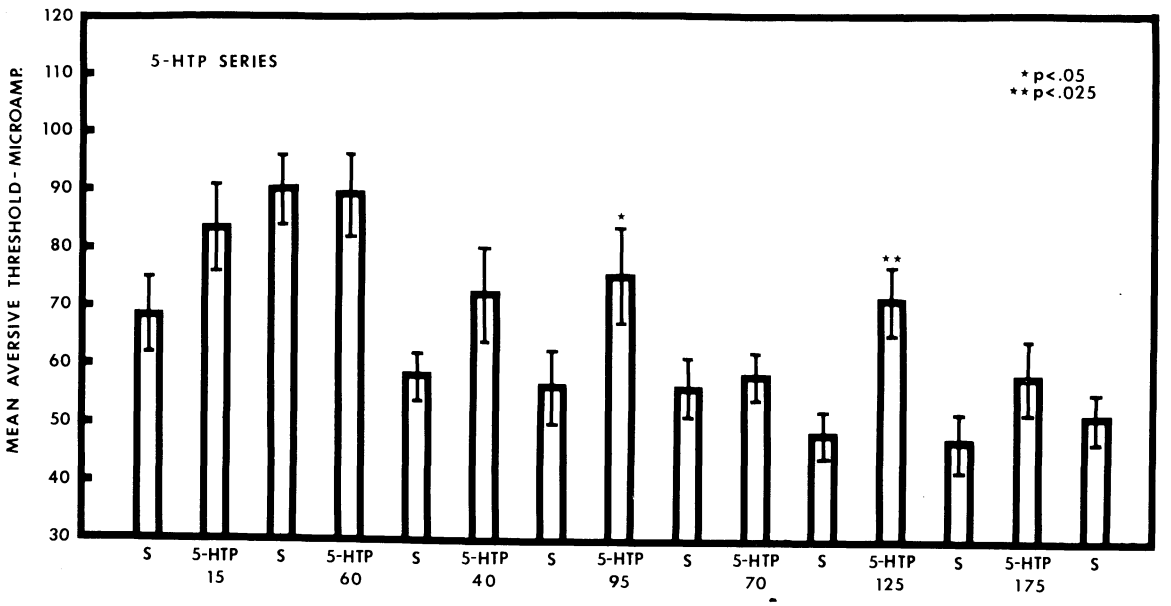

Fig. 1. Mean aversive threshold in microamperes with corresponding standard error of the means for those six animals subjected to various doses of 5-HTP. Each bar represents the mean of three consecutive saline (S) or drug sessions. Probability levels are given for individual F tests comparing the various drug dosages with the preceding saline series. All dosages are given in mg/kg.

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.

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. The number of crossing responses made during each shock intensity was also recorded for each animal.

After 10 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 consecutive weekly series. No drug was administered for the first 3 days of each weekly series (i.e., Monday-Wednesday) followed by 3 days of a particular drug dosage (i.e., Thursday-Saturday). Animals were not tested on the seventh day of these weekly series.

The two drugs administered in the present study consisted of 5-hydroxytryptophan (15, 60, 40, 70, 95, 125, 175 mg/kg) and p-chlorophenylalanine (300, 200, 100, 50 mg/kg). The 5-HTP was dissolved in .9% saline and administered in a volume of 1.0 ml/kg ½ h before threshold testing. Since 5-HTP does not completely dissolve in saline, a small amount of tween 80 was added to make a uniform suspension. P-CPA was prepared in 12.0-ml stock solutions as follows: 1800 mg of p-CPA was dissolved in 4.0 ml of NaOH(5N); the pH was then adjusted to 2.4 by adding 2.0 ml of HCL(5N); then this final solution was diluted with .9% saline to obtain the proper drug concentrations for the various drug dosages. Each dosage of p-CPA was administered in a volume of 2.0 ml/kg in one injection, 24 h before the first drug testing session. Thus, p-CPA was given on Wednesdays in one injection immediately after the third control session of each weekly series. Drug sessions then followed on Thursday, Friday, and Saturday. The p-CPA animals received one injection of the control (i.e., no drug) solution (pH 2.4) on Sundays before the three control sessions (i.e., Monday-Wednesday). All injections were given intraperitoneally.

RESULTS

An analysis of variance (Myers, 1966) performed on the daily thresholds for all doses of p-CPA indicated that no significant differences occurred between control and drug sessions. A similar lack of effect was noted when the number of crossings data were analyzed. Thus, p-CPA in all the doses tested (i.e., 300, 200, 100, 50 mg/kg) had no significant effects on the aversive threshold or the number of crossings made in the spatial preference technique.

The mean aversive threshold data for the six animals subjected to the various dosages of 5-HTP are presented in Fig. 1. As Fig. 1 indicates, only the 95- and 125-mg/kg dosages reliably raised the aversive threshold to moderate levels. The effects noted in this figure were not dose related in that higher doses did not lead to greater increments in the aversive threshold.

Figure 2 presents the mean number of crossing data for those animals subjected to the various dosages of 5-HTP. These data indicated that the middle range of doses (i.e., 60, 70, 95, 125 mg/kg) were all able to reliably reduce the number of crossings made, while the extreme doses (i.e., 15, 40, 175 mg/kg) had no effect. Again, the results in Fig. 2 did not appear to reflect a clear dose-response relationship. In addition to the data in Fig. 1, the six animals who received 5-HTP were given

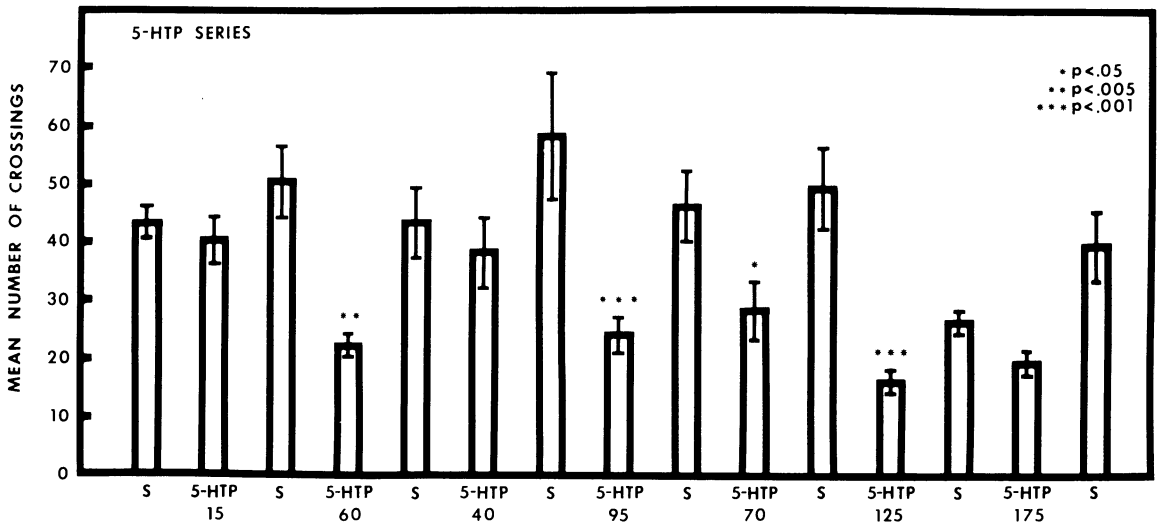

Fig. 2. Mean number of crossing responses with corresponding standard error of the means for those six animals subjected to 5-HTP. Each bar represents the mean of the three consecutive saline (S) or drug sessions. Probability levels are given for individual F tests comparing the various drug dosages with the preceding saline series. All dosages are given in mg/kg.

a final test in which they were pretreated with 200 mg/kg of p-CPA 24 h before the start of a 3-day drug series, in which they received 125 mg/kg of 5-HTP ½ h before threshold testing. This dosage of p-CPA was able to block the increase in the aversive threshold and the reduction in motor activity noted when 5-HTP (125 mg/kg) was administered alone.

DISCUSSION

It is apparent from the above results that reductions in serotonergic activity via the administration of p-CPA do not affect the aversive threshold to electric shock in the rat. Furthermore, the fact that 5-HTP was only able to raise the aversive threshold to moderate levels in a manner that was not dose related, suggests that elevations in serotonergic tone probably do not directly affect pain sensitivity in the rat. The elevations in the aversive threshold noted under 95 and 125 mg/kg of 5-HTP were in all likelihood due to the reductions in motor activity noted under these dosages (e.g., see Fig. 2). These reductions in motor activity, in turn, could have been produced by one or more of the nonspecific effects of the drug. Intravenous injection of serotonin in man has been reported to produce various sensations including nausea and cramps (Hollander, Michelson, & Wilkins, 1957). Animals subjected to these effects of the drug could have been sufficiently debilitated to cause enhanced escape latencies and thus produce higher aversive thresholds. To conclude, it would appear that variations in behavior exhibited in the rat in response to alterations in serotonergic tone in aversive test situations are probably not a reflection of a change in the aversive threshold to electric shock.

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