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L-Tryptophan Decreases the Breaking Point Under a Progressive Ratio Schedule of Intravenous Cocaine Reinforcement in the Rat

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McGREGOR, A., S. LACOSTA AND D. C. S. ROBERTS. *L-Tryptophan decreases the breaking point under a progressive ratio schedule of intravenous cocaine reinforcement in the rat.* PHARMACOL BIOCHEM BEHAV **44**(3) 651–655, 1993.—L-Tryptophan (100 mg/kg, IP), the serotonin [5-hydroxytryptamine (5-HT)] amino acid precursor, significantly reduced the mean breaking point maintained under a self-administration progressive ratio schedule of IV cocaine reinforcement (0.6 mg/injection). This effect was produced over the 5 days of self-administration following treatment. Responding maintained under the same progressive ratio schedule for food reinforcement was not affected by L-tryptophan (100 mg/kg, IP). Rats administered L-tryptophan (100 mg/kg, IP) and denied access to cocaine on the day of treatment resumed normal self-administration patterns under a progressive ratio schedule on following test days. This indicates that L-tryptophan treatment alone did not induce long-term effects on cocaine self-administration. Thus, it would appear that the combination of this 5-HT manipulation and cocaine administration altered the reinforcing efficacy of the drug and induced a long-term decrement in breaking point under a progressive ratio schedule. This may have been due to an associative aversion to cocaine self-administration behaviour learned on the day of treatment and carried over to the subsequent 5 days of self-administration access.

Cocaine L-Tryptophan Self-administration Serotonin Progressive ratio

A dopaminergic role in psychostimulant positive reinforcement is well evidenced. However, more recently interest has also turned to the role of the serotonergic neurotransmitter system. Increasing evidence demonstrates that manipulation of CNS 5-hydroxytryptamine (5-HT) levels alters cocaine and amphetamine self-administration. Both intraventricular and medial forebrain bundle (MFB) 5,7-dihydroxytryptamine (5,7-DHT) lesions, which deplete forebrain 5-HT content, increase amphetamine self-administration (7,9). Conversely, fluoxetine pretreatment (a 5-HT reuptake inhibitor) decreases both amphetamine (7) and cocaine self-administration (2).

Measuring the rate of drug self-administration is open to problems of interpretation (19,21), that is, increasing 5-HT function may enhance the reinforcing efficacy of these drugs, thus reducing the self-administration necessary to achieve the same level of positive reinforcement. Alternatively, 5-HT activity may produce or enhance aversive effects of these drugs, which might also be expected to reduce self-administration. The use of a progressive ratio (PR) schedule of reinforcement allows an alternative method of measuring drug reinforcement efficacy (14).

Some of the effects on psychostimulant self-administration resulting from 5-HT manipulations have been reassessed using a PR schedule. Richardson and Roberts (12) found that fluoxetine reduced the breaking point (BP) reached by animals responding for cocaine reinforcement. Consistent with this result, Loh and Roberts (8) reported that 5,7-DHT lesions of the MFB, which depleted 5-HT levels in the nucleus accumbens, amygdala, and frontal cortex, caused an increase in postoperative breaking point reached under the same PR schedule of cocaine reinforcement.

A role for serotonergic mechanisms in psychostimulant-reinforced behaviour has been further implicated by work that has used L-tryptophan to alter CNS 5-HT levels. Both dietary (3,17) and IP (7) administered L-tryptophan have been found to significantly reduce the self-administration rate of amphetamine and cocaine. Pretreatment with the amino acid tryptophan increases brain levels of tryptophan and serotonin (4,6). Moreover, such an increase in serotonin synthesis results in increased release of the neurotransmitter from nerve terminals in the brain (1,15,16). The experiments reported here extend the work that examined the effects of L-tryptophan on cocaine reinforcement under a fixed ratio (FR) schedule (3,7,17) by examining the effects under a PR schedule.

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METHOD

Animals

Male Wistar rats (Charles River, Quebec), weighing 300–350 g at the start of an experiment, were housed in a temperature-controlled room (21 ± 1°C) under a reversed lighting schedule (lights off 1000–2200 h) with food and water freely available.

Self-Administration

Animals were initially food deprived for 24 h and then trained to press a response lever under a FR 1 schedule of food reinforcement (45-mg Noyes pellet). After two overnight training sessions of over 200 responses, animals were implanted with a chronic jugular cannula while under barbiturate anaesthesia (Somnotol, 60 mg/kg, IP) [see (13) for details].

Animals were individually housed in Plexiglas test cages (25 × 25 × 25 cm) fitted with a removable response lever and a stimulus light above the lever. Drug infusions were delivered from Razel pump-driven 10-ml syringes that were connected via polyethylene tubing (PE50, Portex) to a counterbalanced cannula swivel mounted above the cage. After a 24-h recovery period, animals were trained to press the response lever under a FR 1 schedule of cocaine reinforcement (1.5 mg/kg). Each 0.1-ml injection was delivered over 0.5 s. Once an animal had maintained a consistent response pattern for 3 days, the PR schedule was introduced. To earn a cocaine injection, the response requirement for each successive injection was increased in the following series: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737, 901. The number of reinforcements earned, prior to a 1-h period of nonreinforcement, represented the BP reached in a self-administration session.

During cocaine delivery, the stimulus light above the lever was illuminated and left on for 20 s, signalling a time-out period during which no cocaine could be obtained. All self-administration sessions lasted 5 h and were initiated with a "priming" injection activated by the experimenter.

Animals had to achieve 3 consecutive days of consistent PR performance before being accepted into the study (±2 BP). Similarly, prior to treatment with L-tryptophan or saline the 3 previous consecutive days had to be suitable baseline data (i.e., ±2 BP). Following saline treatment 3 days of data were obtained and following L-tryptophan treatment 6 days of data were obtained before baseline data for the next treatment was collected.

L-Tryptophan (Sigma Chemical Co., St. Louis, MO) was dissolved in distilled water (100 mg/ml) and stored at 4°C. Cocaine HCl (NIDA, Rockville, MD) was dissolved in 0.9% sterile saline (5 mg/ml) and stored at room temperature.

Statistics

All self-administration results were analysed with repeated-measures analyses of variance (ANOVAs) on BP and posthoc

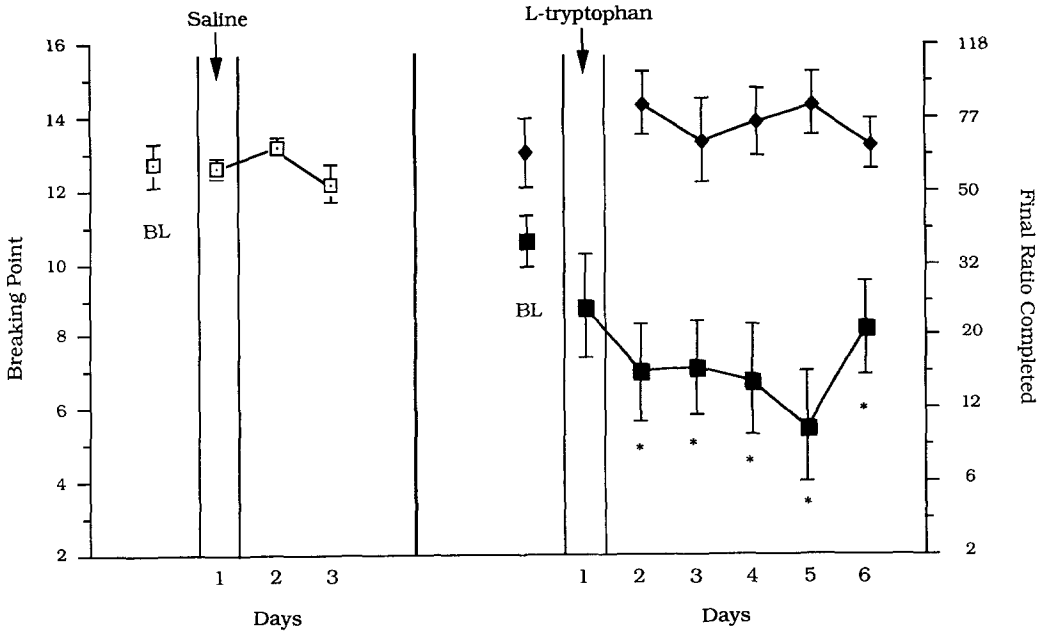

FIG. 1. Mean (±SEM) breaking point (BP) reached under a progressive ratio (PR) schedule of cocaine reinforcement (0.6 mg/injection) following either vehicle (1 ml/kg, IP) (left) or L-tryptophan (100 mg/kg, IP) (right) treatments. Each point represents the mean BP for the group on consecutive days. Day 1 represents the treatment day itself. The left and right panels have been separated to emphasise the counterbalanced design of treatment administration. The final ratio corresponding to the mean BPs is shown on the right axis. Squares (n = 7) represent the results from Experiment 1a, where the pretreatment was administered 1 h prior to cocaine self-administration access. Diamonds (n = 6) represent the results from Experiment 2a, where the pretreatment was administered on a day when cocaine self-administration was made unavailable; cocaine self-administration was only resumed on the day following this treatment. Statistics: posthoc comparison of group means (Student-Newman-Keuls) following analysis of variance; *$p < 0.05$ compared to baseline. BL, baseline; collapsed data for the three consecutive BPs immediately prior to treatment.

comparisons of group means as required (Student-Newman-Keuls).

Experiment 1a: Effect of L-tryptophan treatment on cocaine self-administration

Seven males were trained under the PR schedule of cocaine reinforcement and then administered L-tryptophan (100 mg/kg, IP) or vehicle (1 ml/kg, IP) treatments 1 h prior to the onset of the cocaine self-administration session. Following collection of new baseline data, the animal received the alternate treatment. Four animals received the vehicle treatment followed by the L-tryptophan treatment and three animals received the treatments in the reverse order.

Results. Figure 1 presents the mean BP (±SEM) reached under a PR of cocaine reinforcement following vehicle and L-tryptophan treatments. Three days of baseline data were collected prior to each of the treatments and have been collapsed into a single baseline score for each treatment. No significant difference was found between the two sets of baseline data, $F(5, 25) < 1$, n.s. A one-way ANOVA revealed no significant effect of the vehicle treatment on mean breaking point, $F(3, 15) < 1$, n.s. In contrast, there was a significant effect of the L-tryptophan treatment, $F(5, 25) = 4.6$, $p < 0.05$, with significant decreases produced on test days 2, 3, 4, 5, and 6 following L-tryptophan administration on test day 1 (posthoc comparison of group means; $p < 0.05$).

Experiment 1b: Effect of L-tryptophan in the absence of cocaine on subsequent cocaine self-administration

The results from the first experiment demonstrated that pretreatment with L-tryptophan (100 mg/kg, IP) caused a significant decrease in animals' BPs under a PR schedule of cocaine reinforcement. The question remained, however, as to whether this was a specific effect of the L-tryptophan–cocaine interaction on the first day of treatment or if the L-tryptophan alone produced long-term effects on CNS function that impaired the subsequent reinforcing action of cocaine. Thus, to examine this possibility a second group of animals was trained under the PR schedule and an identical experiment to the previous self-administration study was carried out except on the day of L-tryptophan treatment (100 mg/kg, IP) no access to cocaine was allowed. Animals ($n = 6$) were placed back into the cocaine self-administration routine on the day following this treatment.

Results. The results from this experiment are also presented in Fig. 1. L-Tryptophan pretreatment (100 mg/kg, IP) did not produce any effects on subsequent cocaine self-administration when administered on a day that cocaine was made unavailable to the rat. A one-way ANOVA revealed no significant treatment effect, $F(1, 5) = 1.05$, $p > 0.05$, on the BP reached during the five subsequent self-administration sessions.

Experiment 2: Effect of L-tryptophan on responding under a PR schedule of food reinforcement

A separate group ($n = 8$) of uncannulated rats was used in the food reinforcement study. These animals were housed in pairs and maintained on a 1 h/day ad lib feeding schedule, which always followed the testing session and kept body weights constant. Animals were trained to press a response lever under the PR schedule (see above) of food reinforcement (a 45-mg Noyes pellet). Once a stable baseline of responding had been reached (±2 BP), the animal received its first treatment.

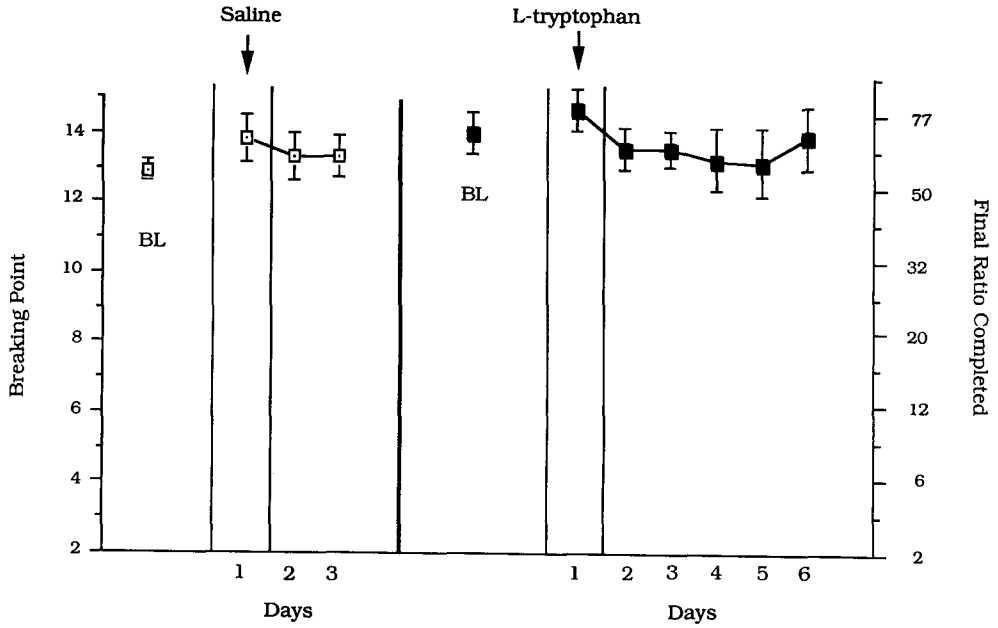

FIG. 2. Mean (±SEM) breaking point breaking point (BP) reached under a progressive ratio (PR) schedule of food reinforcement following both vehicle (1 ml/kg, IP) (left) and L-tryptophan (100 mg/kg, IP) (right) treatment ($n = 8$). The final ratio corresponding to the mean BP is shown on the right axis. Statistics: no significant effect; see the text for details. BL, baseline; collapsed data for the three consecutive BPs immediately prior to treatment; see the text for details.

L-Tryptophan (100 mg/kg, IP) or vehicle (1 ml/kg, IP) were administered 1 h prior to the onset of the food reinforcement session. The treatments were delivered in a counterbalanced fashion.

Results. Figure 2 shows the effect of L-tryptophan (100 mg/kg, IP) or vehicle treatments on the mean (±SEM) breaking point obtained under a PR schedule of food reinforcement. There was no difference between the two treatments' baseline data, $F(5, 35) = 1.02$, $p > 0.05$. ANOVA revealed that neither vehicle, $F(3, 21) = 1.96$, $p > 0.05$, nor L-tryptophan treatment, $F(5, 35) = 2.4$, $p > 0.05$, affected the mean breaking point reached for food reinforcement.

DISCUSSION

L-Tryptophan (100 mg/kg, IP) significantly reduced the BP reached under a PR schedule of cocaine reinforcement. Moreover, the BP remained reduced for the five self-administration sessions subsequent to treatment day (Experiment 1a). This effect was not the result of an aversive effect of the L-tryptophan alone but rather was due to an interaction between the cocaine and the L-tryptophan on the day of treatment (Experiment 1b).

In contrast, responding maintained by food reinforcement under the same PR schedule was not affected by L-tryptophan treatment (100 mg/kg, IP). This indicates that the interference in responding under the PR schedule of cocaine reinforcement did not reflect motor impairments induced by the treatment alone. These results also suggest that the effect on cocaine reinforcement might be specific; however, without further studies this conclusion cannot be drawn. The two reinforcement paradigms are slightly different in that the food reinforcement is supplemented whereas the cocaine is only available during the testing session. This makes direct comparisons between the two paradigms with respect to reinforcement mechanisms more difficult.

This result is in agreement with other studies that investigated the effects of L-tryptophan on psychostimulant self-administration and consistently revealed a decrease in the rate of drug administration following such treatment (3,7,17). The results presented here demonstrate that L-tryptophan pretreatment altered the reinforcing quality of cocaine in a negative direction. As discussed in the introductory section, interpretation of decreased self-administration rate is open to debate, but a decrease in BP on a PR schedule, as seen here, indicates with greater certainty that the reinforcing efficacy of a drug has been attenuated in some way. This effect is in accord with those demonstrating that fluoxetine, a 5-HT reuptake inhibitor, decreases BP (12) and 5-HT lesions of the forebrain increase BP under this schedule of cocaine reinforcement (8).

However, as can be seen from Fig. 1, the effect on BP following L-tryptophan treatment was relatively long lasting. Such an effect has been noted in previous studies examining 5-HT involvement in psychostimulant reinforcement (2,3, 17,22), although none report such a prolonged delay in the return to normal levels of responding following treatment (see below). Given that systemic injection of L-tryptophan (100 mg/kg, IP) causes an increase in brain levels of serotonin that peak by 1 h and a return to control levels by 4 h, the reduction in cocaine reinforcement cannot be accounted for by a long-term change in serotonin levels (4,6). The long-term effects of L-tryptophan treatment reported here may reflect an aversive conditioning effect on cocaine self-administration following an aversive experience of cocaine self-administration on the day of L-tryptophan treatment. Experiment 2 revealed that it was indeed an interaction between L-tryptophan and cocaine that induced these long-term effects on self-administration and not the result of L-tryptophan action alone. The reason for the prolonged effects on cocaine self-administration behaviour reported here, in comparison to the lesser effects in other studies using L-tryptophan treatment (3,17), is unclear. However, if the interpretation of an aversive conditioning effect is correct the less severe conditioning in the other studies may have resulted from the route of L-tryptophan administration. Both Smith et al. (17) and Carroll at al. (3) used dietary L-tryptophan supplements to manipulate 5-HT levels in the CNS. This treatment may prevent strong conditioning effects by inducing a gradual increase in the levels of 5-HT and therefore in the aversive nature of the interaction with cocaine. Such a nondiscrete aversive event might not support aversive conditioning to drug self-administration behaviour as strongly as a single L-tryptophan injection. Lecesse and Lyness (7) used a route of administration and dose of L-tryptophan similar to that employed in the present study and reported a decrease in the rate of amphetamine intake only during the first 4–5 h of the test session. However, they employed 8-h testing sessions and found that the levels of drug intake recovered in the second half of the test session following L-tryptophan treatment. Allowing animals access to the drug after 4–5 h, when the levels of 5-HT in the CNS would have fallen again, may have ensured an immediate reestablishment of the positive effects of drug intake and thus prevented any long-term aversive conditioning effects to self-administration behaviour.

Although the results demonstrate that an interaction between L-tryptophan and cocaine reduced the reinforcing efficacy of cocaine, they do not directly address the issue, discussed above, of aversive conditioning being responsible for the long-term effects on self-administration behaviour.

These results lend support to the hypothesis that 5-HT-mediated mechanisms may underlie an aversive aspect of psychostimulant drug action [see (17)]. Amphetamine and cocaine have been shown to induce aversive effects under certain behavioural test conditions (5,18,20). Thus, during normal self-administration conditions the positively reinforcing effects of such drugs, mediated by dopamine action, may dominate the control of drug intake, but when 5-HT action is enhanced an aversive component of drug action may become unmasked.

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