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Dopamine Agonists Prevent or Counteract the Suppression of Brain Stimulation Reward by Fenfluramine

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OLDS, M. E. *Dopamine agonists prevent or counteract the suppression of brain stimulation reward by fenfluramine.* PHARMACOL BIOCHEM BEHAV **50**(1) 41-48, 1995.—The interaction between the serotonin (5-HT) and the dopamine (DA) systems in the modulation of intracranial self-stimulation (ICSS), a DA-dependent behavior, was investigated. Chronically implanted rats for ICSS in the medial forebrain bundle were tested for the effects of fenfluramine at a dose of 20 mg/kg, and then for the effects of 10 mg/kg piribedil plus 2 mg/kg amphetamine, injected 30 min before fenfluramine or 60 min after fenfluramine. Our aim was to determine whether the action of fenfluramine at the DA binding site could be blocked by prior occupation, or whether if it were occupied by fenfluramine it could be reversed. Fenfluramine, 20 mg/kg, injected alone, suppressed ICSS for 5-7 h. The suppression was followed by a prolonged recovery during which ICSS was profounded depressed. Repeating the treatment 7 days later produced the same response, except that the suppression was of shorter duration. In another group of animals, pretreatment with piribedil plus amphetamine 30 min before fenfluramine prevented the suppression of ICSS. Instead, ICSS was briefly attenuated, then restored to baseline levels, and then facilitated. Repeating the treatment 7 days after the first treatment potentiated this response. The attenuation was now even briefer, the recovery more rapid, and the facilitation more robust. In still another group of animals, fenfluramine was given just before the ICSS session began. Predictably, the effect was a total cessation of ICSS. At 60 min into the session, piribedil plus amphetamine was injected. The response showed a rapid recovery of ICSS followed by facilitation. Repeating the treatment 7 days later potentiated this response. Recovery to baseline ICSS was more rapid and facilitation was larger and longer lasting. These results are discussed in terms of a possible neuroleptic-like action of fenfluramine being responsible for a portion of the effects of fenfluramine on ICSS, which suggests a basis for the protection afforded by the DA agonists.

Amphetamine Dopamine Intracranial self-stimulation Fenfluramine Piribedil Serotonin

FENFLURAMINE is a substituted amphetamine that shares with the parent compound the ability to reduce appetite, but not the ability to induce behavioral stimulation (12,34,35). Interest in this compound is based on the notion that it may be valuable in the treatment of feeding disorders without incurring the liability associated with amphetamine, and may prove useful in the study of serotonin (5-hydroxytryptamine, 5-HT) functions because of its actions on 5-HT transmission (34). The actions on activity in the 5-HT systems are both short- and long-term, and are considered primary, but actions on other systems have been reported (34).

The biochemical data for the serotonergic actions of fenfluramine show that it has the ability to induce a massive release of 5-HT centrally, as evidenced in striatum and other regions of the forebrain innervated by the 5-HT neurons of the midbrain (4,11,19,30,34,35,40,46). This release has been shown to be followed by depletion of central 5-HT levels when the supply for release is exhausted and synthesis fails to keep up with release (34,46). Functionally, it can be assumed that the release of 5-HT leads to enhanced serotonergic activity in the short run, and that the depletion leads to depressed serotonergic activity in the long run. In situations in which fenfluramine was administered to animals at high doses, the depletion of 5-HT levels was shown to be permanent because it resulted from damage inflicted to 5-HT terminals, and thus to the 5-HT storage mechanism (1,14,20,21,36,37). There have been many attempts to correlate these actions of fenfluramine on 5-HT transmission with the effects of the drug on feeding, motor activity, and other functions associated with serotonergic activity, but the efforts have been only partially successful (34).

The biochemical data supporting the notion that fenfluramine has the ability to influence dopaminergic transmission show that it produces the release of dopamine (DA) in the short run and, in that time frame, causes an increase in the levels of DA metabolites in striatum (3,6,8-10,13,17,18,34,

46). However, fenfluramine is not toxic to DA neurons; therefore, the depletion that might follow the release would be temporary, even if the drug were given at a high dose. The increase in DA metabolites induced by fenfluramine was shown to be preventable by pretreatment with piribedil, a direct DA agonist, but not by pretreatment with a 5-HT antagonist (17,18,46). The findings related to DA metabolites have been interpreted to mean that the high levels represent increased turnover aimed at overcoming a blockade of the DA receptor by fenfluramine acting directly at that site. This interpretation has led to the notion that the action of fenfluramine is neuroleptic-like in character, in view of the similarity between this response and that evoked by treatment with neuroleptics.

The effects of fenfluramine on behaviors known to depend on the integrity of the brain DA systems support the notion that fenfluramine has a neuroleptic-like action. Thus, it has been shown to have the capacity to depress motor activity without inducing sedation, and to counteract the motor activity induced by amphetamine and apomorphine (2,3,15,19, 27,34). Furthermore, it was shown that amphetamine and apomorphine can counteract the depressed motor activity induced by fenfluramine (34); but these findings point mainly to the neuroleptic-like action of fenfluramine on transmission in the nigrostriatal DA system, not on transmission in the mesolimbic or mesocortical systems (7,16,24). However, results obtained with fenfluramine on intracranial self-stimulation (ICSS) suggest that its action extends to the mesolimbic system (23,30).

Intracranial self-stimulation is currently viewed as a DA-dependent behavior according to evidence that procedures that enhance dopaminergic activity facilitate ICSS, and those that interfere with it depress ICSS (43–45). Early on, however, ICSS was associated with catecholaminergic activity, and serotonergic activity was believed to depress ICSS through its negative control of catecholaminergic activity (3,32,33). The current view is that serotonergic activity still regulates ICSS, but rather through its modulation of the output of the midbrain DA neurons giving rise to the mesolimbic system associated with ICSS. Because fenfluramine has both serotonergic and dopaminergic actions, and ICSS is a DA-dependent behavior, a determination of the basis for the effects of fenfluramine on ICSS would provide additional evidence for the neuroleptic-like action of fenfluramine.

Fenfluramine had been shown to depress ICSS (23), but the basis for this effect was not investigated. In a follow-up study of these early findings, we determined the long-term effects of fenfluramine given at a high dose to induce permanent depletion of 5-HT levels (30). The results showed that fenfluramine given at a dose of 20 mg/kg induced a suppression of ICSS that lasted from 13–24 h, and that recovery extended over several days. We also showed that repeating the treatment at weekly intervals ascerbated these effects until ICSS could no longer be obtained. Given the time limits of the actions of fenfluramine on activity in the 5-HT and the DA systems (46), it seemed interesting to ascertain which aspects of its effects on ICSS were due to the serotonergic actions and which to the dopaminergic actions. The strategy was the same as that used to demonstrate the capacity of the DA agonist piribedil to block the increase in DA metabolites induced by fenfluramine in striatum (9,10,17,34,46). The specific purpose was to determine whether preempting occupation by fenfluramine of the DA receptor by pretreatment with a DA agonist, or a combination of DA agonists, would prevent the long-term suppression and depression of ICSS associated with fenfluramine.

METHODS

The preparation, behavioral protocols, and analysis of the data were the same as in our previous study of the effects of fenfluramine on ICSS (30).

Subjects

The subjects were adult male, Sprague-Dawley-derived rats weighing 300–350 g. Before electrode implantation and during the 7–10 days of recovery that followed, the animals lived in groups of three in colony cages. During ICSS training and testing the animals lived in their individual operant chambers. Eight animals were tested concurrently in sessions that started at 1800 and stopped at 0700. After each session, the electrode connector was removed, and a rest period followed until the next session began. Food and water were available on demand during the rest and ICSS sessions. The temperature in the experimental room was set to 23°C. The lights in the room were set to go off at 1800 and come on at 0700 to signal to the animal the beginning and end of the ICSS session.

Electrodes and Implantation Procedures

Each rat was implanted with one bipolar stimulating electrode in the medial forebrain bundle (MFB) using conventional stereotaxic techniques (22). The electrode was a pair of twisted, insulated stainless-steel wires 250 μm in diameter, threaded into a nylon pedestal permanently attached to the skull. The electrode, connector, and the implantation methods were the same as those used in our previous study (30). Briefly, the implantation involved the following steps: The animal was anesthetized with pentobarbital (50 mg/kg, IP), following which its head was fixed in a rat stereotaxic instrument. The skin over the skull was retracted, and the skull was cleaned to expose bregma and lambda. Several small holes were then drilled in the skull, one of which used stereotaxic coordinates (22) selected to be over the MFB; the others were placed randomly in a circle a few millimeters away from the MFB hole. The coordinates used for the MFB were 3.5 mm posterior from the bregma, 1.5 mm lateral to the midline, and 8.3 mm from the top of the skull. Stainless-steel anchoring screws were placed in the other holes. After the electrode was lowered to the appropriate depth, acrylic dental cement was spread around the base of the pedestal and over the anchoring screws. After the cement had cured, the skin was brought around the base of the pedestal and sutures were placed where appropriate. The animal was then removed from the stereotaxic instrument and placed in a rodent breeding cage for recovery from the anesthesia. A recovery period of 8–10 days was allotted before training to ICSS began. During that time, the implanted animals lived in groups of three in rodent colony breeding cages, with food and water available on demand.

Apparatus and Training

All training and drug testing took place in the dark period of the rats' 24-h circadian cycle, set in this experiment to start when the lights in the experimental room were turned off, and to terminate when the lights were turned on 13 h later.

The operant chamber was made from Plexiglas and measured 17 × 40 × 33 cm. It was fitted with a pedal placed on the front panel about 1.5 cm above the Plexiglas floor, which separated the chamber from a pan filled with wood shavings. The chamber was also fitted with a counterbalanced overhead arm that carried the leads from the stimulator. Food and water were available on demand during the training and test sessions.

Each depression of the lever led to a train of 60-Hz sine

waves being applied for 0.25 s to MFB. Pressing the lever during the application was ineffective in producing a new train. Training to ICSS consisted in giving a series of 10 consecutive nightly sessions, with the current intensity set at 60 μA in the first session; it was then raised in each successive session by 20 μA until ICSS criteria were met or the maximum of 160 μA had been made available to the animal when it pressed the lever. When ICSS criteria were met, current intensity was not raised further. When the maximum current intensity was reached, it remained at that level until the end of the 10 training sessions. At that time, the animals that failed to meet ICSS criteria were removed from the study. Drug tests started in the 11th session given the next night. The ninth and 10th training sessions were preceded by injections of 0.9% saline given IP.

The ICSS criteria required a lever-pressing rate of 10,000 or higher per session and an even distribution of lever-pressing throughout the session. This pattern of response rate had to be repeated from session to session as an indication that it was stable for the animal at that intensity.

The experiments were performed under the control of a DEC PDP-11 34. A computer that collected, stored, and analyzed the responses made in a session. The scores were made available to the experimenter in the morning when the session finished. A hard copy of the scores for each animal was made available, showing the total number of responses made in the session and the response rate per 10 and 60 min over the course of the session. Group data were calculated on the basis of the hourly rate.

Drugs

The drugs used were DL-fenfluramine HCL (Sigma, St Louis, MO), piribedil mesylate (Laboratoires Servier, Paris France), and D-amphetamine sulfate (Sigma). Fenfluramine was given at the high dose of 20 mg/kg to induce the release of 5-HT in the short-term and to cause a depletion of 5-HT levels in the long-term (12,14,20,21,26,30,37,39,41). This high dose was also selected with the expectation that it would lead to a robust increase in DA metabolites in the short-term, as reported in some of the biochemical studies of the action of fenfluramine on DA metabolism (15,20,46). Finally, the high dose of fenfluramine was selected to replicate the long-term depressant effects of fenfluramine on ICSS that we reported previously (29,30), in an attempt to determine whether protection can be afforded by enhanced dopaminergic activity.

Piribedil, a direct DA agonist, was given as one of the pretreating agents in this study because it had been used to prevent the increase in DA metabolites that followed the injection of fenfluramine (6). In this study, we injected piribedil 30 min before fenfluramine to achieve the same end—the binding of piribedil to the DA receptor—and thus, presumably to reduce accessibility of the DA receptor to binding by fenfluramine. To ensure maximal reduction of accessibility, we decided to combine the injection of piribedil with the indirect DA agonist amphetamine (27). We thus hoped to saturate the DA binding sites yet not interfere with this process because of competition between piribedil and amphetamine. The release of catecholamines by amphetamine (27) was assumed to make free DA available if binding by piribedil was not sustained long enough to overlap with fenfluramine, or if it did not stimulated enough DA receptors. Amphetamine was given at a dose of 2 mg/kg because of evidence from our previous use of this drug on ICSS that it reliably induced a robust facilitation (30). Thus, the pretreatment before fenfluramine was a combination of piribedil plus amphetamine, and the posttreatment after fenfluramine was the same combination, but here it was given 60 min after fenfluramine, when ICSS was suppressed.

The drugs were freshly mixed before each ICSS session, and were injected IP immediately before the session began. Fenfluramine was dissolved in a few drops of 0.3% tartaric acid brought up to volume with 0.9% saline, and piribedil was dissolved in a few drops of chloroform brought up to volume with 0.9% saline. When the protocol called for piribedil plus amphetamine as the pretreatment, these drugs were injected, but the ICSS session started only 30 min later, after fenfluramine was injected. When the protocol called for piribedil plus amphetamine as the posttreatment, fenfluramine was injected first, then the ICSS session started to establish that the drug had its usual effect, and 60 minutes later, piribedil plus amphetamine was given. The interruption in the ICSS session for the fenfluramine injection was minimal. There was no evidence at that time that the animal was sedated or showed unusual behavior, even though ICSS was suppressed. For the posttreatment, the animal was removed from the operant chamber for 1–2 min and the injection was given, after which the animal was returned to its chamber and to the ICSS session. The effects of saline or saline containing 0.3% tartaric acid were determined in the last two sessions of training.

Analysis of the Data

The hourly scores of the last four ICSS training sessions, including the two preceded by the injection of the vehicle, were used to compute the baseline ICSS hourly rates (mean ± SD) for each drug-naive animal. These were then used to compute baseline group ICSS hourly scores (mean ± SD). Group test scores were computed from the hourly scores achieved in the drug session, and the differences between them and the baseline scores were evaluated for statistical significance using one-way analysis of variance (ANOVA) for repeated measures, followed by Student's t test where appropriate (42). A value of $p < 0.05$ was taken as the minimal value for statistical significance.

When a second treatment was given to the same animals, the test scores were compared with new baseline scores. These were computed from the hourly rates achieved in daily sessions 5 and 6 of the 7-day interval between two treatments. They represented the group baseline ICSS rates in drug-experienced animals in the two sessions preceding the second drug test (mean ± SD).

Histology

After completion of each experiment, the animals were overdosed with pentobarbital. The brains were removed, placed in formalin, and sectioned by the frozen technique 8–10 days later. Placement of the probe was determined from cresyl violet-stained sections. The reported results were obtained in animals in which the probes were in the MFB.

RESULTS

Fenfluramine in Drug-Naive and Fenfluramine-Experienced Rats

Figure 1 shows the results obtained with fenfluramine. The top panel shows the effect in drug-naive animals ($n = 5$). The baseline ICSS rate was relatively high, fluctuating between approximately 1700–2000 presses/h. Injecting fenfluramine blocked ICSS for 5 h. This was followed by a weak and protracted recovery yielding an ICSS rate in the range of 500–700/h during the next 8 h of the session (Fig. 1, top panel).

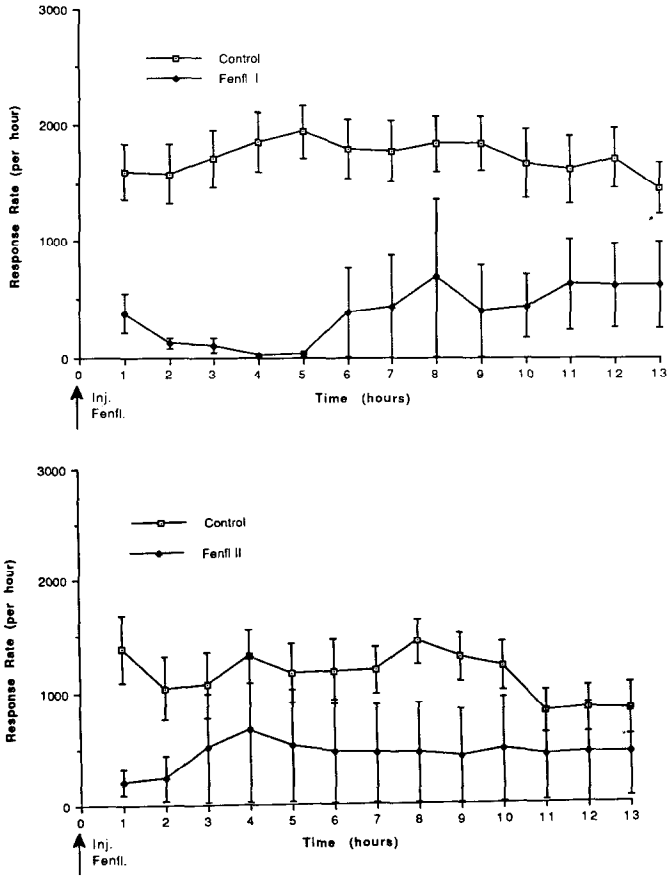

FIG. 1. Effects of fenfluramine (Fenfl) 20 mg/kg IP on intracranial self-stimulation (ICSS). Top panel: Effects of the drug in drug-naive animals (n = 5). Bottom panel: Effects of the drug injected (Inj.) a second time, 7 days later. The first treatment initially blocked ICSS and then depressed it; the second treatment depressed ICSS throughout the session.

Giving fenfluramine a second time to some of these animals (n = 3), 7 days later, when ICSS had recovered (but not to the initial predrug state), blocked ICSS for 2 h instead of 5. (Fig. 1, bottom panel). The blockade was followed by a weak and protracted recovery yielding an ICSS rate in the range of 500-700/h for the next 8 h. Fenfluramine thus retained its capacity to block ICSS, albeit for a shorter duration. The depression of ICSS that followed the blockade was of the same magnitude as that seen after the first-time treatment.

Piribedil Plus Amphetamine Before Fenfluramine

The injection of piribedil plus amphetamine in drug-naive animals, and 30 min later, the injection of fenfluramine followed immediately by the ICSS session, led to a triphasic drug response. ICSS was depressed at first, but not suppressed, as it was after fenfluramine given alone, and peak effect was seen in the second hour into the session. This was followed by a relatively rapid return to the baseline ICSS rate in the fourth hour of the session and remained there for a period. This was followed by facilitation of ICSS, which attained peak effect in the 10th hour and remained at that level for 3 h when the session ended (Fig. 2, n = 6, piribedil plus amphetamine 30 min before fenfluramine I). Evaluation of the data with one-way ANOVA for repeated measures revealed that the total number of lever presses during the control and test sessions did not differ significantly, yet the scores were significantly different when time was taken into consideration (the control scores remaining fairly constant over the 13-h session, whereas the drug session scores fluctuated between early depression and facilitation of ICSS (F = 3.81, df = 12, $p < 0.001$). The analysis also showed an interaction between treatment and time, yielding a significant difference between the two sets of scores (F = 6.2, df = 12, $p < 0.001$). Thus, the pretreatment prevented the long-term suppression of ICSS, substituting instead a relatively brief attenuation of the behavior, and delayed for several hours the facilitation of ICSS usually seen after the injection of DA agonists.

This treatment was repeated 7 days later. The pretreatment was now even more effective in blunting the action of fenfluramine on ICSS. Not only was ICSS not blocked, as it was when fenfluramine was given alone, but it was not even deeply depressed, as it was after the first pretreatment. ICSS was now mildly attenuated for a much briefer time than after the first pretreatment. In addition, the facilitation occurred earlier, without the prolonged period when ICSS was restored to control levels; it was also more robust and lasted from the second or the third hour of the session until its end 10 h later (Fig. 3, n = 6, piribedil plus amphetamine 30 min before fenfluramine II). There was no difference in the total number of responses between the control and test sessions, but there was a significant difference when time was taken into consideration (F = 8.67, df = 12, $p < 0.001$). Treatment and time interacted to yield a significant difference between the two sets of scores (F = 6.65, df = 12, $p < 0.001$). Thus, in the drug-experienced animals, pretreatment with the DA agonists was more effective than in the drug-naive animals in blocking the effects of fenfluramine on ICSS. In addition, the ability of fenfluramine to delay the facilitation of ICSS induced by the DA agonists on ICSS was further reduced compared with the results seen in the drug-naive subjects.

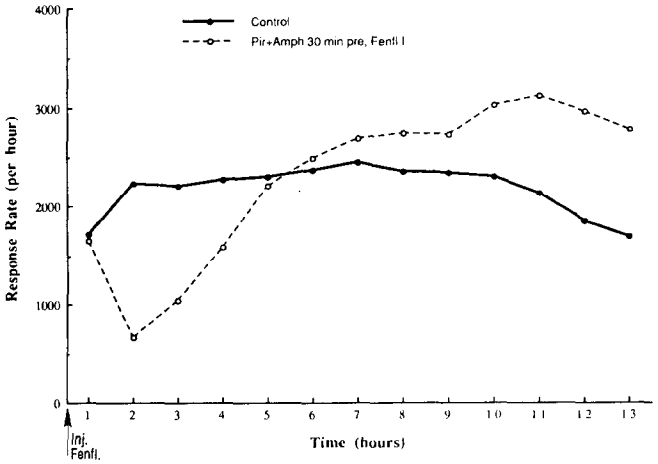

FIG. 2. Effects of pretreatment with dopamine agonists on the intracranial self-stimulation (ICSS) response to fenfluramine in drug-naive rats (Pir + Amph 30 min pre, Fenfl I). Injecting (Inj.) piribedil 10 mg/kg plus amphetamine 2 mg/kg IP 30 min before fenfluramine 20 mg/kg given IP did not block, but only attenuated ICSS for 3 h, and then induced a prolonged facilitation (n = 6). The pretreatment protected against the blockade and long-term depression of ICSS seen with fenfluramine given alone.

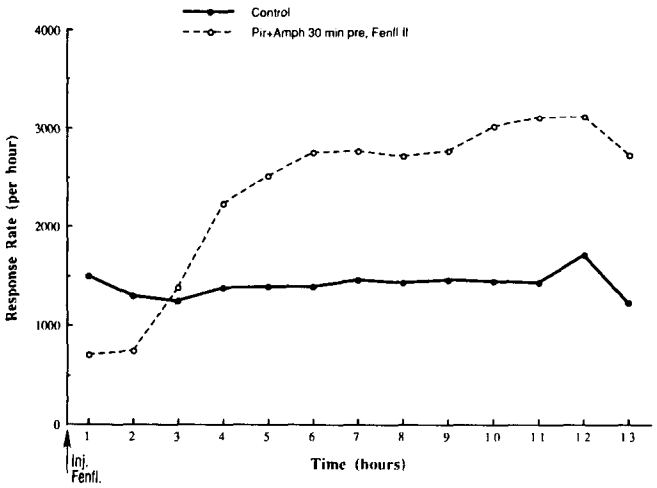

FIG. 3. Effects of repeating the treatment with dopamine agonists given before fenfluramine (Pir + Amph 30 min pre, Fenfl II). Injecting (Inj.) piribedil 10 mg/kg plus amphetamine 2 mg/kg IP 30 min before fenfluramine 20 mg/kg IP in the drug-experienced rats (n = 6) increased the protection afforded by the dopamine agonists against the blockade and depression of intracranial self-stimulation (ICSS) induced by fenfluramine. ICSS was attenuated for a briefer period, recovery was more rapid, and facilitation was more prolonged.

Piribedil Plus Amphetamine After Fenfluramine

The aim in this experiment was to determine whether the blockade of ICSS by fenfluramine at peak effect could be antagonized by injecting the animals with piribedil plus amphetamine. Therefore, the session started with 30 min of control ICSS in drug-naive animals. At the end of this period, they were injected with fenfluramine and the session continued for another 60 min. At that time, the subjects were injected with piribedil plus amphetamine and the session continued until its end 11.5 h later.

In the 30-min control period that started the session, the ICSS response rate was in the range of the ICSS rate in the control sessions (Fig. 4, n = 6, fenfluramine I 60 min before piribedil plus amphetamine). In the next 60 min ICSS was suppressed, reflecting the ability of fenfluramine, given 30 min into the session, to block ICSS. At the end of the 60 min, when piribedil + amphetamine was injected, ICSS was still blocked, but a gradual period of recovery was started, as shown by ICSS occurring in the third hour of the session. This was followed by facilitation of ICSS extending from the fourth to the eight hour, and then by a return to the baseline rate, lasting until the end of the session 5 h later. Although the total number of responses in the control and drug sessions did not significantly differ, the number of responses made at different times over the course of the session differed (F = 2.48, df = 12, $p < 0.006$), and treatment and time interacted also to result in a significant difference between the two sets of scores (F = 2.98, df = 12, $p < 0.001$). Thus, the blockade of ICSS induced by fenfluramine in the drug-naive animals was shown to be capable of being antagonized by the injection of the two DA agonists. However, the negative effect of fenfluramine on ICSS lingered, as shown by the relatively modest facilitation induced by the DA agonists.

This experiment was repeated 1 week later in the same animals, now drug experienced. The response was dramatically altered (Fig. 5, n = 4, fenfluramine II 60 min before piribedil plus amphetamine). ICSS was at the control level in the first 30 min before any drug was given. After fenfluramine, given at the end of the 30-min control period, ICSS was only mildly attenuated, in sharp contrast to the blockade of ICSS seen 1 week earlier when fenfluramine had been given. When the combination of piribedil + amphetamine was injected 60 min after fenfluramine, ICSS was more rapidly restored to the baseline rate and then more rapidly and more robustly facilitated for the next 10 h than in the drug session given 1 week earlier. There was no difference in the total number of responses made in the control and test sessions, but the control and test scores differed significantly over time (F = 3.21, df = 12, $p < 0.001$). There was also a significant interaction between treatment and time (F = 3.36, df = 12, $p < 0.0006$). Thus, in the drug-experienced animals, the effectiveness of the two DA agonists to antagonize the suppression of ICSS was potentiated and their capacity to facilitate ICSS was enhanced.

Effects of Amphetamine and Piribedil Plus Amphetamine

Amphetamine injected in drug-naive animals produced a facilitation of ICSS (Fig. 6, top panel). Peak effect occurred between 30 and 120 min after the injection, but shortly afterward this effect decreased, and ICSS was restored to the baseline rate. However, it did not stay at this level, but instead became attenuated and remained so for several hours. This phase was in turn followed by a return of ICSS to the baseline rate, where it stayed until the end of the session. Thus, amphetamine induced a rapid and dramatic increase in ICSS that was of relatively short duration in relation to the session.

Piribedil plus amphetamine, given to drug-naive animals, produced effects essentially similar to those seen with amphetamine alone (Fig. 6, bottom panel). The ICSS rate increased dramatically within a few minutes after the injection of the two DA agents, reached peak effect within the first hour, and then declined rapidly, with control level being reached in the fifth hour of the session. The ICSS rate stayed at that level or fell below it in the period between the fifth hour and the end of the session.

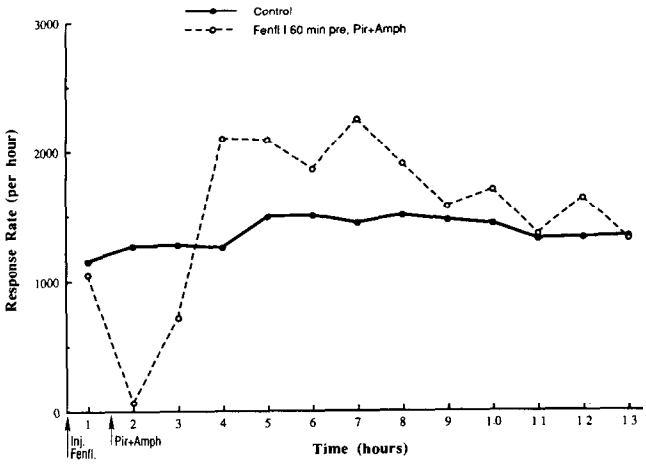

FIG. 4. Effects of the dopamine agonists given during fenfluramine-induced blockade of intracranial self-stimulation (ICSS) (drug-naive rats, Fenfl I 60 min pre, Pir + Amph). Fenfluramine, 20 mg/kg IP, blocked ICSS. The administration of piribedil, 10 mg/kg plus amphetamine, 2 mg/kg IP 60 min later, led to a rapid recovery of ICSS and then to its facilitation (n = 6).

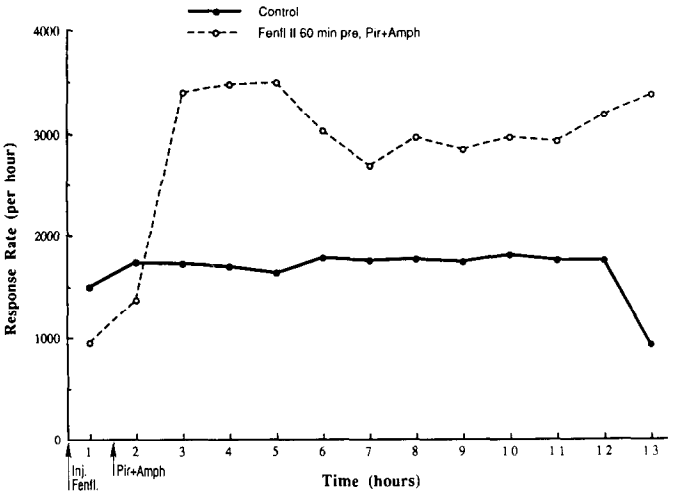

FIG. 5. Effects of repeating the treatment (7 days later) with fenfluramine given initially, then, 60 min later, the DA agonists (Fenfl II 60 min pre, Pir + Amph). Fenfluramine, 20 mg/kg, followed later by piribedil, 10 mg/kg, plus amphetamine, 2 mg/kg, (n = 6), led to the same response as that seen for the first time treatment. In the drug-experienced animals, however, fenfluramine did not block intracranial self-stimulation (ICSS) but only attenuated it, and the DA agonists were more effective in restoring ICSS and inducing its facilitation.

The experiments thus show that the psychostimulant, given alone, produced a quick and dramatic increase in ICSS that did not last, and that the combination of piribedil plus amphetamine produced essentially the same effect, with the two drugs not showing synergism.

DISCUSSION

The data obtained with fenfluramine injected alone confirm our previously reported findings (30). In both studies, fenfluramine given at the 20 mg/kg dose abolished ICSS within a few minutes of its administration. However, in our previous study, the suppression lasted throughout the session, also of 13 h, whereas here the suppression lasted 5–7 h. It is likely that the difference was due to differences in the baseline rate of ICSS; the higher the rate, the shorter the period when ICSS was abolished. Nevertheless, all animals showed suppression of ICSS, and in all animals the effect was seen for several hours. None of the animals was sedated or failed to move about the chamber. There was no obvious dysfunction in the appearance of the animals, which over the 13 h session engaged in reduced, yet normal activities, except that they moved away from the lever and only occasionally returned to press it. The long-term suppression of ICSS followed by its long-term depression is interpreted to mean that the actions of fenfluramine on serotonergic activity played a role in its effects on ICSS because these effects were long-term, as were the effects on ICSS. This interpretation is consistent with the current view that the serotonin neurons exert inhibitory regulation of DA neuronal output and as a result inhibitory regulation of ICSS (25,31–33).

The results of the two experiments testing for the capacity of piribedil plus amphetamine given 30 min before fenfluramine, to prevent the fenfluramine-induced suppression of ICSS show that such protection was achieved. Suppression was not seen, but nevertheless, fenfluramine affected ICSS, as shown by the delay of the DA agonists-induced facilitation of ICSS. Instead, shortly after the administration of fenfluramine, ICSS was attenuated. This effect is viewed as reflecting in the drug-naive animals the release of 5-HT by fenfluramine, but not its binding to the DA receptor already occupied and stimulated by free DA and piribedil. The findings thus show that enhanced 5-HT activity leads to a short-term attenuation of ICSS, but not to its long-term suppression. For suppression to occur, the serotonergic action of fenfluramine has to synergize with its neuroleptic-like action.

The results of a second treatment revealed an increased capacity of the DA agonists to blunt the negative effects of fenfluramine on ICSS. The attenuation observed shortly after the administration of fenfluramine was milder and of shorter duration than after the first treatment, and the recovery to baseline ICSS was more rapid. This difference may be explained as resulting from less 5-HT available for release in these animals, in which a portion of 5-HT terminals had been destroyed because of the high dose of fenfluramine given, as indicated by depleted 5-HT levels in forebrain serotonergic terminal fields observed in our previous study (30). The findings of the pretreatment experiments thus underscore the fact that the effects of only altering 5-HT activity on ICSS are

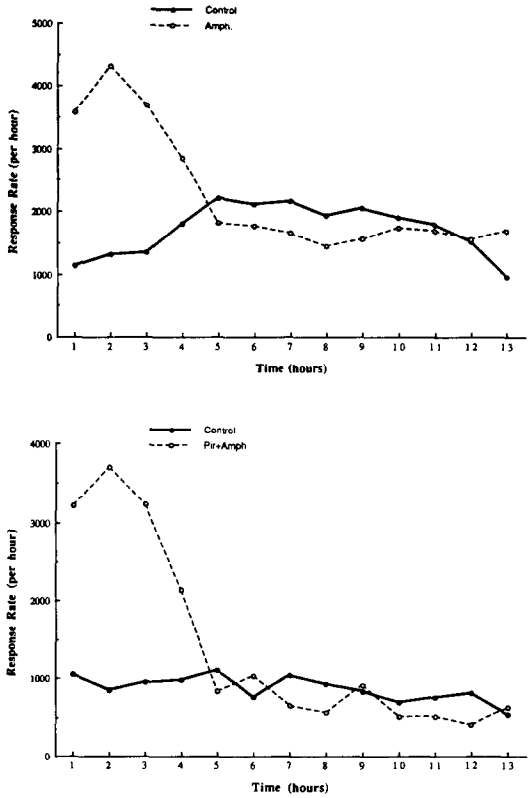

FIG. 6. A comparison of the effects of amphetamine given alone and of piribedil plus amphetamine injected at the same time. Amphetamine, 2 mg/kg IP, given to drug-naive subjects led to a fourfold facilitation of intracranial self-stimulation (ICSS) with peak effect attained in the second hour of the session (top panel, n = 6). ICSS then decayed rapidly to below baseline ICSS. Injecting piribedil 10 mg/kg plus amphetamine 2 mg/kg led to a response of similar magnitude and duration (n = 6).

modest; but the findings also show that they can be potentiated by altering dopaminergic activity in an appropriate manner. It thus appears that the two systems interact at the level of the reward function via their regulation of dopaminergic activity, but that the nature of the action on DA activity is the primary factor determining whether ICSS will be abolished or only attenuated.

The results of the experiments in which the DA agonists were given after fenfluramine show a suppression of ICSS during the 60 min preceding the injection of the DA agonists, as had been expected from previously reported results from this laboratory (30), and confirmed in the present study. In addition, the present results show that the suppression can be counteracted within a short time after the administration of the two DA agonists injected simultaneously. The response to the posttreatment was a rapid restoration of ICSS followed by a prolonged facilitation. This capacity to counteract the actions of fenfluramine is interpreted to reflect a change in the state of the DA receptor and in the level of activity of the 5-HT system. However, because in this experiment the DA agonists were given after fenfluramine, the state of the DA receptor and the activity of the 5-HT system when the DA agonists were administered, were different from the state and activity present in the pretreatment experiment.

It seems reasonable to assume that 5-HT was released during the 60 min following the administration of fenfluramine (34,35,39), and that during that same period there was an increase in DA metabolites, reflecting the blockade of the DA receptor, as indicated by the results of biochemical studies of the actions of fenfluramine on dopaminergic activity (3,34). After 60 min, however, release of 5-HT is likely to have diminished because synthesis may not have kept up with massive release. According to the evidence, the release of DA by fenfluramine and its action at the DA binding site is transient, and is therefore likely to have been in the process of restoration to baseline. Under such conditions, DA released by amphetamine and by the brain stimulus applied in the MFB, as well as by piribedil, might have had only limited access to the DA binding site. Inhibitory regulation by the 5-HT neurons of ICSS, on the other hand, was likely to diminish as a result of depletion of 5-HT stores. These conditions were presumed to provide the appropriate environment for DA agonists injected 60 min after fenfluramine to be effective in partly counteracting the suppression of ICSS by fenfluramine. When these two actions of fenfluramine ceased, ICSS was facilitated.

The results of the repeat experiment in the same animals show improved ability of the DA agonists to antagonize the suppression of ICSS induced by fenfluramine given 60 min earlier. The explanation offered here is the same as that offered for the improved capacity of the DA agonists given before fenfluramine to prevent the suppression of ICSS—namely, not to a change in the extent of the neuroleptic-like action of fenfluramine at the DA receptor, but to depressed 5-HT transmission resulting from fenfluramine-induced damage of 5-HT terminals. This had a disinhibiting effect on the DA neurons once the neuroleptic-like action had run its course; in that environment, the DA agonists became highly effective in facilitating ICSS, as shown by a more robust and longer-lasting facilitation than seen in the fenfluramine-naive rats.

The explanation for some of the effects of fenfluramine on ICSS in terms of its biochemical actions—in part, its capacity to induce the release of 5-HT and, at the high dose here used, permanently to lower 5-HT levels (11,12,34,35,39–41) finds support in the existing literature. Similarly, the explanation for some of the other effects of fenfluramine on ICSS in terms of its capacity to depress dopaminergic activity by a direct action at the DA binding site (3,27,34) finds support in the literature. The evidence shows that DA agonists can counteract the depression of motor activity in rats induced by fenfluramine, and that fenfluramine can counteract the hyperactivity induced by amphetamine and apomorphine (2,15,19,34). Apparently, the relationship between 5-HT and DA activity presumed to exist for the regulation of drug-induced motor activity extends to the regulation of brain stimulation-induced reward. This explanation is consistent with the view that serotonergic transmission exerts inhibitory regulation of behaviors, some of which, like ICSS, have been shown to be DA-dependent (2,19,23,25,28,33,35,38).

The results support the notion that fenfluramine has a neuroleptic-like action, and that blocking this action leads to an attenuation of the negative effects of fenfluramine on ICSS. However, because a portion of these effects remained and because the facilitation by DA agonists was delayed, it must be assumed that the serotonergic actions of fenfluramine could not be altogether overcome, not even for a DA-dependent behavior such as ICSS.

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