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The Anxiogenic Agents, Yohimbine and FG 7142, Disrupt the Noradrenergic Response to Novelty

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MASON, K., D. J. HEAL AND S. C. STANFORD. *The anxiogenic agents, yohimbine and FG 7142, disrupt the noradrenergic response to novelty.* PHARMACOL BIOCHEM BEHAV **60**(2) 321–327, 1998.—Whether or not abnormal noradrenergic transmission can be a causal factor in anxiety is controversial. The present experiments examined this question by comparing the effects of two anxiogenic agents on noradrenaline efflux in the frontal cortex of freely moving rats. A single anxiogenic dose of either yohimbine (2.5 or 5 mg/kg) or FG 7142 (10 or 20 mg/kg) was administered to rats by IP injection. Yohimbine increased spontaneous efflux of noradrenaline, but FG 7142 had no effect. However, subsequent exposure of rats to a novel environment increased noradrenaline efflux in vehicle-, but not drug-treated rats. Calculation of the net change in noradrenaline efflux caused by transfer to the novel environment showed that this was reduced by yohimbine, whereas FG 7142 increased it. These two compounds also had different effects on locomotor activity in the novel environment. The results suggest that anxiety is unlikely to be invariably associated with increased noradrenergic transmission, in the frontal cortex at least. However, it remains possible that any disruption of the noradrenergic response to stress could be an underlying feature of anxiety. © 1998 Elsevier Science Inc.

FG 7142 Microdialysis Noradrenaline Novel environment Yohimbine Stress

EXPERIMENTS using microdialysis in vivo have shown that stressful stimuli, with a prominent somatosensory component, increase the concentration of extracellular noradrenaline in rat brain [e.g., immobilization: (18); footshock: (31); tailpinch: (29)]. Furthermore, even exposure of rats to a novel environment (6), or conditioned cues for an aversive stimulus (31), can have the same effect. Such findings suggest that an increase in central noradrenergic transmission is a key component of the response to psychologically aversive stimuli as well as those that incur physical discomfort.

It is also widely believed that increased release of noradrenaline in the brain is an underlying cause of anxiety. This is not least because of the many similarities between anxiety and the stress response (23). To some extent, this proposal is borne out by human studies (4), but evidence from animal models for anxiety is equivocal. Many studies have investigated the effects of the α_2-adrenoceptor antagonist, yohimbine, on rats' behavioral response to psychological stimuli based on "novelty." This drug, which increases noradrenaline release by acting as an antagonist at presynaptic α_2-autoreceptors, modifies rats' behavioral response to novelty in a manner consistent with an anxiogenic effect (13,14). However, it is doubtful whether α_2-adrenoceptor antagonism explains this behavioral change (16,22,30). A further complication is that, when using animal models based on behavioral reactions to "conflict," this drug has an anxiolytic action (17). These disparate findings echo the differences in the effects of 5-HT in behavioral tests based on novelty and conflict (12).

One striking feature of all this work is that the effects of anxiogenic drugs on behavior are evaluated during exposure of rats to aversive environmental stimuli. In contrast, the neurochemical effects of these agents have been characterized in unstressed animals. The possibility that anxiogenic drugs disrupt, or exaggerate, the noradrenergic response to novel stimuli has not been examined. The present experiments investigated this possibility using microdialysis of the frontal cortex

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in freely moving rats. The effect of yohimbine on noradrenaline efflux in rats kept in their home cage was compared with that in rats exposed to a novel environment. Also, to test whether the changes induced by yohimbine generalize to other anxiogenic agents, the same experiments were carried out using (*N*-methyl-β)-carboline-3-carboxamide (FG 7142). This benzodiazepine inverse agonist can induce profound anxiety both in humans (9) and in animal models of anxiety in which novelty is the key feature (5,10).

These studies exposed marked differences in the effects of yohimbine and FG 7142 on spontaneous efflux of noradrenaline and on the noradrenergic response to novelty. It is concluded that either an increase in noradrenergic transmission is not a consistent effect of anxiogenic drugs or that any disruption (either an excess or a deficit) in the noradrenergic response to stress could account for a behavioral response that indicates anxiety.

METHOD

Subjects

All procedures complied with the UK Animals (Scientific Procedures) Act, 1986. Outbred male Sprague–Dawley rats (260–350 g), derived from a colony at University College London, were used. Animals were transferred to their housing cages, in groups of four, at least 1 week in advance of experiments. Rats were allowed unrestricted access to food and water and their environment was maintained at 22°C with a 12L:12D cycle (lights on 0700 h). On the day of surgery, rats were transferred to individual "home cages" (diameter 260 mm) to which they were returned for overnight recovery.

Surgery and Microdialysis

Microdialysis probes, constructed in this laboratory [see (6)], were vertically implanted in the frontal cortex (sterotaxic coordinates: AP +3.5 mm; ML ± 1.5 mm; DV −5.5 mm) (19) under halothane anaesthesia. On the following day (i.e., 17–20 h postsurgery), the probes were perfused at 1.0 μl/min with Ringer's solution of the following composition (mM): NaCl 145; KCl 4; $CaCl_2$ 1.3; pH 6.6. The first 80 min of dialysate was discarded after which samples were collected, at 20-min intervals, into 5 μl of 0.01 M perchloric acid. The position of the microdialysis probe was verified histologically at the end of each experiment.

Neurochemical Analysis

The concentration of noradrenaline in the dialysis samples was determined using reverse phase HPLC with electrochemical detection (Coulochem model 2100 A; ESA). Separation was achieved by an analytical column (Hypersil; 5 ODS C18 250 × 4.6 mm) using a mobile phase comprising (mM) sodium dihydrogen orthophosphate 83, sodium octanesulphonic acid 2.77, EDTA 0.85 and 12% methanol, adjusted to pH 3.4 with orthophosphoric acid.

Procedure

After collection of four consecutive dialysis samples, which were used to assess the spontaneous ("basal") efflux of noradrenaline, rats were injected with either yohimbine (2 or 5 mg/kg IP), FG 7142 (10 or 20 mg/kg IP) or an equivalent volume of their respective vehicles (2 ml/kg IP). Yohimbine hydrochloride was dissolved in distilled water (3 mg/ml) and diluted to volume in saline (0.9% w/v NaCl). FG 7142 was suspended in distilled water by adding 1 drop of Tween 80 and sonicating before the addition of distilled water.

After spending 1 h in their home cage, rats were either transferred to a novel arena or remained in their home cage for the rest of the experiment. The novel arena consisted of a circular enclosure (diameter 39 cm; height 31 cm), which was divided into quarters by lines drawn on the base; these enabled locomotor activity to be scored. An unfamiliar object (cotton reel) was placed in the center of the enclosure to add to the novel features of the arena (8). The enclosure was illuminated at the same intensity as the animals' home cage (300 lx). Rats were placed in the arena for a period of 2 h and their behavior recorded by a videocamera, mounted directly above the apparatus. Locomotor activity was scored as total lines crossed in each of six consecutive 20-min time bins. The criterion for scoring was that both the rat's head and front paws crossed a line.

Statistical Analysis

The noradrenaline content of the dialysis samples was expressed as fmol/20 min without correction for recovery. Differences in efflux at different points of the time course were compared across bins of data comprising three consecutive samples per bin. This was so as to distinguish relatively prolonged changes, which could underlie any anxiogenic drug effects, from transient effects arising from the injection, for instance. Only the last three samples of spontaneous ("basal") efflux were included in the first bin. The significance of changes in efflux was evaluated using split-plot ANOVA (SPSS PC^+) with "time (within each bin)" or "bin" as "within subjects" factors and "environment" as the "between subjects" factor. Net changes in efflux, caused by exposure to the novel environment, were calculated and analyzed using split-plot ANOVA as described in (7). Finally, drug-induced changes in locomotor activity were analyzed by split-plot ANOVA. In all cases, the criterion for statistical significance was $p < 0.05$.

Drugs and Reagents

Halothane ("Fluothane"; Zeneca) was purchased from the pharmacy at University College Hospital. Yohimbine HCl was purchased from Sigma Chemical Co. (UK), and FG 7142 was a gift from Schering A/C Berlin. *l*-Arterenol bitartrate, used as an external standard for the HPLC, and octanesulphonic acid were purchased from Sigma Chemical Co. (UK). All other reagents for the mobile phase of the HPLC comprised AnalaR grade chemicals purchased from BDH.

RESULTS

Spontaneous Efflux of Noradrenaline

Despite an apparent rise in the baseline after IP injection of saline or Tween vehicle, any increase in efflux was transient, only: neither treatment had any significant overall effects on efflux of noradrenaline in the rat frontal cortex either in the first hour or the subsequent 2-h postinjection (Figs. 1a and b). In contrast, administration of yohimbine (2 mg/kg IP) caused an approximately twofold increase in efflux of noradrenaline (Fig. 1a). This increase was statistically significant during the first, $F(1, 10) = 13.9$, $p = 0.004$, second, $F(1, 9) = 18.7$, $p = 0.002$, and third, $F(1, 7) = 13.0$, $p = 0.009$), hour after injection. The higher dose of yohimbine (5 mg/kg IP) caused even larger increases in efflux which reached concen-

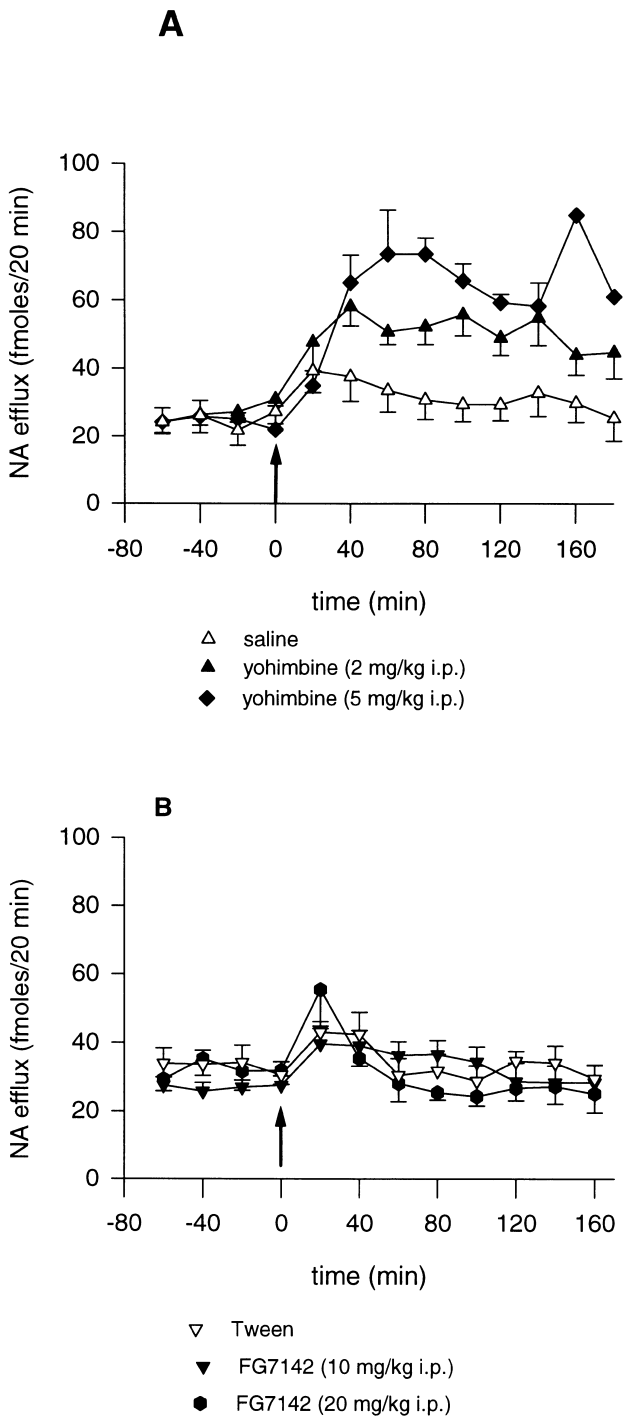

FIG. 1. The effects of (A) yohimbine (2 mg/kg IP, $n = 7$ or 5 mg/kg IP, $n = 3$) or saline (2 ml/kg IP, $n = 7$), and (B) FG 7142 (10 or 20 mg/kg IP, $n = 5$) or Tween vehicle (2 ml/kg IP, $n = 5$) on the efflux of noradrenaline in the frontal cortex of freely moving rats maintained in their home cage. Yohimbine, FG 7142, or vehicle were injected at time "0" (marked with arrow). Points indicate mean ± SEM.

trations threefold greater than basal during the third hour postinjection (Fig. 1a). In contrast, neither dose of FG 7142 (10 or 20 mg/ kg IP) increased noradrenaline efflux in either the first hour after injection, or subsequently (Fig. 1b).

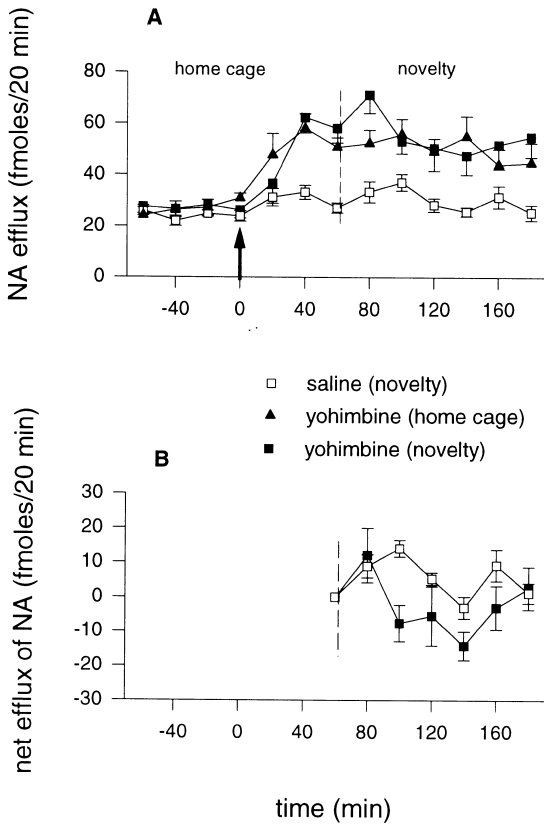

FIG. 2. (A) The effects of yohimbine (2 mg/kg IP) or saline (2 ml/kg IP) on the efflux of noradrenaline in rat frontal cortex during exposure to a novel environment; rats were injected at time "0" (marked by arrow) and remained in their home cage for 1 h. They were then either transferred to a novel arena (both groups: $n = 7$), or remained in their home cage ($n = 7$) for a further 2 h (indicated by bar). (B) Net change in the efflux of noradrenaline in yohimbine- and saline-pretreated rats during exposure to a novel environment. Points indicate mean ± SEM.

Effect of Yohimbine (2 mg/kg IP) on the Efflux of Noradrenaline During Exposure to a Novel Environment

As before, injection of saline did not significantly increase efflux of noradrenaline during the first hour postinjection (Fig. 2a). However, on subsequent transfer to the novel arena, efflux of noradrenaline was approximately 35% greater than in the basal samples for the first hour, $F(1, 11) = 5.8$, $p = 0.035$. This increase in efflux was not sustained and was no longer evident during the second hour in the novel arena.

When compared with the basal samples, yohimbine significantly increased the concentration of noradrenaline in the dialysis samples collected both while animals were in their home cage, $F(1, 11) = 16.1$, $p = 0.002$, and during the 2-h exposure to the novel environment [first hour: $F(1, 11) = 20.7$, $p = 0.001$; second hour: $F(1, 10) = 6.19$, $p = 0.03$)] (Fig. 2a). However, there was no difference in the efflux of noradrenaline in yohimbine-pretreated rats that were transferred to the novel environment and those that remained in their home cage (Fig. 2a).

To assess whether yohimbine modified the noradrenergic response to novelty, the net change in noradrenaline efflux, caused by exposure to the novel arena, was calculated (see the Method section). This procedure compensates for any under-

lying change in baseline caused by the drug alone or any residual effects of the injection. Between 20–100 min in the novel environment, the net efflux of noradrenaline was significantly less after yohimbine pretreatment than in saline-pretreated rats, $F(1, 11) = 7.97$, $p = 0.017$.

Effect of FG 7142 (10 mg/kg IP) on the Efflux of Noradrenaline During Exposure to a Novel Environment

In this batch of rats, noradrenaline efflux significantly increased during the first hour after injection with Tween vehicle, $F(1, 8) = 11.8$, $p = 0.01$. Efflux in the novel arena was also significantly greater than in basal samples during the first, $F(1, 8) = 9.7$, $p = 0.01$, but not the second hour (Fig. 3a).

After injection of FG 7142, noradrenaline efflux did not change while the rats remained in the home cage (Fig. 3a). However, after transfer to the novel cage, noradrenaline efflux increased significantly during the first, $F(1, 9) = 7.37$, $p = 0.02$, but not the second hour (Fig. 3a). Despite this increase, the difference in the concentration of noradrenaline in FG 7142-pretreated rats that were transferred to the novel arena and those that remained in their home cage was not statistically significant. Nevertheless, a specific effect of exposure to the novel arena during the first hour is indicated by a statistically significant treatment × time interaction, $F(2, 16) = 8.3$, $p = 0.003$.

To investigate whether there were any specific effects of FG 7142 on the noradrenergic response to a novel environment, changes in net efflux during novelty were calculated. Again, this would exclude any residual changes in efflux caused by the injection of drug or vehicle alone. Because of the changes in efflux in the Tween-pretreated rats that remained in their home cage, the effects of FG 7142 on the response to novelty are emphasized by calculation of the net efflux of noradrenaline on exposure to the novel cage. Between 20–100 min in the novel arena, FG 7142 significantly increased the net efflux of noradrenaline when compared with that in time-matched Tween (vehicle)-injected controls, $F(1, 8) = 8.37$, $p = 0.02$ (Fig. 3b).

Effects of Yohimbine and FG 7142 on Locomotor Activity in the Novel Cage

In all treatment groups, locomotor activity diminished within the first hour in the novel arena (Fig. 4). Notwithstanding this reduction, locomotor activity was significantly greater in yohimbine-treated rats than in saline controls during both the first, $F(1, 17) = 7.90$, $p = 0.012$, and second hour, $F(1, 17) = 7.30$, $p = 0.015$, in the novel cage (Fig. 4a). In contrast, FG 7142 reduced the locomotor activity during the first hour, when compared with that of Tween-injected rats, $F(1, 13) = 4.78$, $p = 0.05$, but there was no appreciable activity in either treatment group during the second hour (Fig. 4b). In both cases, drug effects on locomotor activity of rats during the first hour in the novel cage were most evident during the first 20 min of exposure.

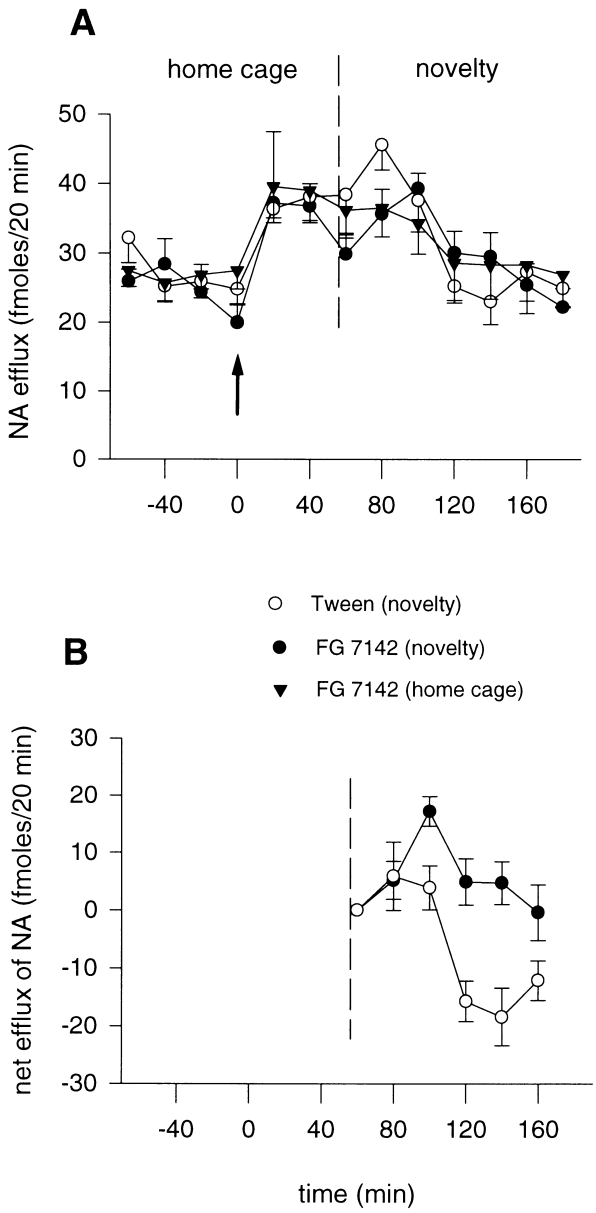

FIG. 3. (A) The effects of FG 7142 (10 mg/kg IP) or Tween vehicle (2 ml/kg IP) on the efflux of noradrenaline in rat frontal cortex during exposure to a novel environment. Rats were injected at time "0" (marked by arrow) and remained in their home cage for 1 h. They were then either transferred to a novel arena (FG 7142, $n = 6$; Tween: $n = 5$), or remained in their home cage (both groups, $n = 5$) for a further 2 h (indicated by bar). (B) Net change in the efflux of noradrenaline in FG 7142- and Tween-pretreated rats during exposure to a novel environment. Points indicate mean ± SEM.

DISCUSSION

The long-standing theory that excessive noradrenergic transmission can be a causal factor in anxiety (23) could well explain the anxiolytic actions of clonidine or β-adrenoceptor antagonists. However, several studies, especially those based on rats' behavioral response to "conflict," have questioned this assertion. In particular, they challenge the view that α_2-antagonists, which increase noradrenaline release in the brain, invariably cause behavioral changes indicative of a state of anxiety (11,24). α_2-Antagonists have more consistent anxiogenic effects in rodent models where novelty is the prominent stimulus. Even so, it is doubtful whether the change in behavior can be attributed solely to increased noradrenergic transmission (16).

An alternative way of investigating this question is to see whether drugs that induce an anxiogenic response to novelty consistently increase the concentration of extracellular noradrenaline. On this basis, the present experiments began by

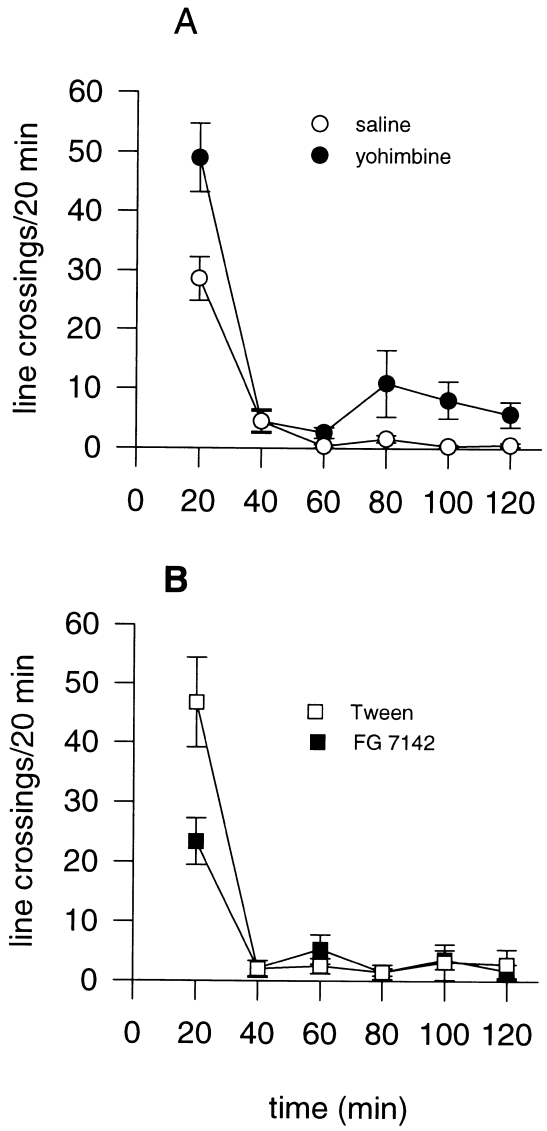

FIG. 4. The effects of (A) yohimbine (2 mg/kg IP, $n = 10$) and saline (2 ml/kg IP, $n = 8$), or (B) FG 7142 (10 mg/kg IP, $n = 9$) and Tween vehicle (2 ml/kg IP, $n = 6$) on locomotor activity during exposure to a novel arena. Points indicate mean ± SEM.

using microdialysis in the frontal cortex of the freely moving rat to compare the effects of yohimbine and FG 7142 on noradrenaline efflux. Doses of yohimbine (13,21,26) and FG 7142 (10,20) were used, which were within the range of those widely reported to evoke an anxiogenic response to novelty.

When tested in rats that remained in their home cage, anxiogenic doses of yohimbine caused a marked increase in noradrenaline efflux in the frontal cortex. This result is consistent with findings from earlier studies of the effects of this drug in the frontal cortex (28), the hippocampus (1), and hypothalamus (15). The magnitude of the increase paralleled the dose of yohimbine. However, the increase was somewhat larger, and was evident at lower drug doses than in either the hippocampus or hypothalamus. In contrast, neither dose of FG 7142, both of which were within the anxiogenic range, increased efflux of noradrenaline. This result confirms that from our previous study (27) but contrasts with its effects on dopamine in this brain region in which it causes a pronounced increase in dopamine efflux (2,3). There is one report of an increase in noradrenaline efflux in the medial prefrontal cortex after a 20 mg/kg dose of FG 7142 (18). Reasons for these disparate results are unknown. Nevertheless, the present results indicate that, when the effects of yohimbine and FG 7142 are evaluated under identical experimental conditions and in doses that are anxiogenic in rats, these drugs have quite different effects on noradrenaline efflux.

Despite these findings, it is still possible that increased noradrenergic transmission could underlie the anxiogenic effects of these drugs. One important factor to consider is that, when extrapolating from neurochemical measurements to explain the neurochemical basis of behavior, these two measures should be carried out under the same conditions. Yet, evaluation of the behavioral effects of anxiogenic drugs inevitably rests on testing rats' response to aversive (e.g., novel) stimuli. In contrast, neurochemical studies of drug action usually aim to eliminate extraneous stressful stimuli. The question arises as to whether yohimbine and FG 7142 might have similar effects on the noradrenergic response to novel environmental stimuli, despite differing in their effects on spontaneous efflux of noradrenaline? In certain respects, this mirrors the findings from human studies: there are several reports that yohimbine induces panic attacks more consistently in patients with an underlying anxiety disorder than in normal subjects (25).

To examine this possibility, the effects of yohimbine and FG 7142 on noradrenaline efflux were tested in animals that were transferred to a novel environment. However, neither drug affected the absolute efflux of noradrenaline in rats transferred to the novel cage when compared with that in animals that remained in their home cage. Yet previous experiments have exposed a marked increase in noradrenaline efflux in drug-free rats when these are transferred to a novel environment (6). Importantly, this finding was replicated in the present study. This finding alone suggests that both yohimbine and FG 7142 might have modified the noradrenergic response to novelty. This is supported by the treatment × environment interaction in FG 7142-pretreated rats, despite the absence of a main effect of drug treatment. Such an interaction was not evident in yohimbine pretreated rats, although a ceiling effect for yohimbine can be excluded. This is because the dose used in this part of the study produced a submaximal increase in noradrenaline efflux in animals that remained in their home cage.

The effects of yohimbine and FG 7142 on the noradrenergic response to novelty were explored in greater detail by evaluating their influence on the net change in efflux caused by transfer to the novel environment. This analysis, which accounts for changes caused, for example, by vehicle injection and which have been noted before (7), revealed appreciable differences in the effects of these two drugs: whereas yohimbine attenuated the net increase in noradrenaline efflux caused by novelty, FG 7142 increased it. Because this analysis corrects for any underlying or residual effects on spontaneous efflux caused by the injection of drug or vehicle [see (7)], it is unlikely that the different effects of FG 7142 and yohimbine are explained by differences in baseline at the onset of exposure to the novel environment.

Interestingly, these two drugs also had opposite effects on locomotor activity during the first hour, and especially during the first 20 min of exposure to the novel environment. One complication is the different locomotor activity scores in the

two control groups: reasons for this difference are unknown. Notwithstanding this disparity, it remains evident that locomotor activity was increased by yohimbine and/or reduced by FG 7142. These results concur with those from previous studies of locomotor activity in the plus-maze (20,26). Obviously, locomotor activity is not a measure of anxiety. However, this finding does provide independent evidence that two supposedly anxiogenic drugs can evoke different responses to the same stimulus, be it neurochemical or behavioral. In this respect, the present findings mirror those from an earlier study of the effects of anxiolytic agents on the noradrenergic response to novelty. This showed that anxiolytic doses of buspirone and diazepam had different effects on spontaneous efflux of noradrenaline but neither drug modified the noradrenergic response to stress (7).

In summary, anxiogenic drugs do not consistently increase spontaneous efflux of noradrenaline or exaggerate the stress response. These results suggest that either the concentration of extracellular noradrenaline has no bearing on the cause(s) of anxiety or that anxiety arises from any inappropriate noradrenergic response to stress, be it either excessive or inadequate. Whether or not this is the case, the present findings do not support the possibility that increased noradrenergic transmission, in the frontal cortex at least, is an invariable cause or consequence of anxiety.

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