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Studies on the role of ACTH and of 5-HT in anxiety, using an animal model

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The effects of ACTH (5 & 7·5 μg/100 g) were studied using a new animal model of anxiety. ACTH had an anxiogenic effect that was maximal during a 10 min test period starting 3 min after injection. The behavioural effects of ACTH were counteracted by chronic administration of chlordiazepoxide (5 mg kg^{-1} for 5 days) and by acute administration of ethanol (0·4 g kg^{-1}). These anxiolytic drugs decreased the turnover of 5-HT in the midbrain, hypothalamus and cerebral cortex, whereas ACTH increased 5-HT turnover in the midbrain and hypothalamus. It is therefore proposed that anxiety results from the action of ACTH, possibly on 5-HT pathways in the midbrain and hypothalamus.

Adrenocorticotrophic hormone (ACTH) has been reported to have a variety of behavioural effects, many of which can be attributed to an increase in motivation (DeWied, 1977a,b). The purpose of these studies was specifically to investigate whether 'physiological' doses of ACTH might have an anxiogenic effect. Experiment 1 was designed to investigate this, using an animal model of anxiety, and to plot the time course of this action of ACTH. These studies were extended in Experiment 2, and in addition we investigated whether the behavioural effects of ACTH could be counteracted by anxiolytic drugs. Experiment 3 was designed to test the hypothesis that a reduction in anxiety results from a decrease in the turnover of 5-HT (Stein, Wise & Berger, 1973), and to investigate whether ACTH might also affect 5-HT turnover.

The animal model of anxiety used is that developed by File & Hyde (1977) in which the time spent by pairs of male rats in active social interaction is measured under various test conditions. Social interaction is highest when the rats are tested in a box with which they are familiar and in a low level of illumination, and decreases if the box is unfamiliar or if the light level is increased. We have shown that the decrease in interaction is not due to an increase in exploration of an unfamiliar environment, nor to changes in olfactory cues from the partner. Drugs with an anxiolytic action produce a constant level of social interaction across all the test conditions (i.e. they prevent the decrease that occurs in control animals) (File, Hyde & Pool, 1976).

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EXPERIMENT 1

TIME COURSE OF ACTION OF ACTH

Methods

Animals. Male hooded rats (*Rattus norvegicus*) from Olac Ltd (Bicester) were housed singly for 5 days before the start of the experiment. During this period they were handled and weighed daily and at the start of testing they weighed 200–250 g. The rats were maintained in a 13 h light: 11 h dark cycle, with lights on at 0700 h. The position of each rat's cage in the rack was changed daily to equate experience of different levels of illumination. Food and water were freely available.

Apparatus. The test box was 66 × 66 cm with a wooden floor and walls 47 cm tall. Infrared cells placed in the walls of the box provided an automated measure of motor activity, a count being scored each time a beam was interrupted. The level of illuminance was 23·5 scotopic lux (scotopic units are appropriate because the rat has a predominantly rod retina).

Drug. ACTH (tetracosactrin, CIBA) was dissolved in 0·9% saline to a concentration of 0·025 mg ml^{-1}. The doses of ACTH were chosen to mimic the plasma corticosterone concentration that would be present during moderate to severe stress (Hodges & Mitchley, 1971).

Procedure. Pilot studies in the low light familiar test condition had shown that when the social interaction test was started 3 min after ACTH injection there was a large decrease in social interaction. If the test

was started 30 min after injection the decrease in social interaction was not significant. On the basis of these results we chose to start testing 3, 15 and 30 min after ACTH injection, and in particular to make planned comparisons between ACTH and saline injected controls at 15 and 30 min.

A total of 108 rats were thus randomly allocated to the following 9 drug groups, 6 pairs being tested in each group and no pair of animals being tested on more than one occasion: saline controls and rats injected with 5 or 7·5 μg/100 g ACTH and tested 3, 15 or 30 min after intraperitoneal injection.

In this experiment all the rats were tested in the low light familiar test condition. To familiarize the rats with the test box they were placed singly in the box for 10 min on 2 consecutive days before the social interaction test. In this test both members of a pair received the same drug treatment. Pairs were tested in a random order between 0800 and 1100 h, during which time there is minimal fluctuation in endogenous corticosterone, and hence presumably in ACTH concentrations (Hodges, 1970).

For the social interaction test each pair of rats was placed in the box for 10 min and their behaviour observed on a television monitor. The time they spent in active social interaction was scored by two observers, one of whom had no knowledge of the drug state of the animals. The inter-observer agreement was within 10 s. The following behaviours were included in the active interaction score: sniffing, following, grooming, mounting, nipping, kicking, boxing, wrestling, jumping on and crawling under or over the partner. Time spent sitting or lying with their bodies in contact was not included in the active interaction score but was scored separately as passive contact. At the end of the 10 min session the rats were removed from the box, any boluses removed and the floor and walls of the box wiped with detergent and dried.

Results

In each group the s.e.m. was approximately 5% of the mean, the scores were normally distributed and there were no significant differences between the variances of the different groups. Therefore parametric statistics were appropriate and the data were analysed by analysis of variance in which the drug treatment was one factor and the time between injection and test was the second factor.

There was a significant reduction in social interaction following injection of ACTH (5 μg/100 g) ($F(1,30) = 27·3$, $P < 0·001$), which was most marked 3 min after injection (see Fig. 1). Planned comparisons revealed that the reduction was still significant 15 min after injection ($F(1,30) = 7·7$, $P < 0·01$), but was no longer significant at 30 min ($F(1,30) = 3·8$). Similar results were obtained with the higher dose of ACTH, which significantly reduced social interaction ($F(1,30) = 6·9$, $P < 0·02$), most markedly at 3 min; by 30 min the reduction was no longer significant.

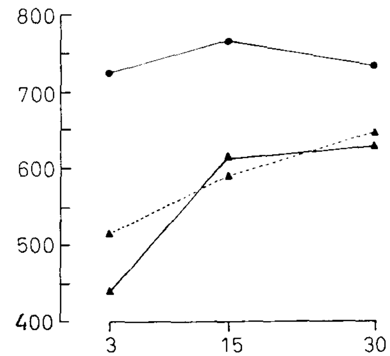

FIG. 1. Time course of effects of ACTH on social interaction in pairs of male rats. ●——● Saline controls, ▲– – –▲ ACTH (5 μg/100 g), ▲——▲ ACTH (7·5 μg/100 g). The abscissa shows the time interval (min) between injection and the start of a 10-min test; the ordinate shows the mean time (min) spent in active social interaction by each group of rats.

The mean motor activity score of the control animals was 688·3 and that for the ACTH injected rats was 719·7: thus there was no evidence from these scores that ACTH had a sedative action, and therefore the decreased social interaction was not the result of any general sedation resulting from ACTH.

EXPERIMENT 2—THE INTERACTION BETWEEN ANXIOLYTIC DRUGS AND ACTH

To demonstrate an anxiolytic action it is necessary to test the animals for social interaction in more than one test condition. Control animals show a decrease in social interaction if the test box is unfamiliar or if the light level is increased. Anxiolytic drugs produce significantly less decrease in social interaction as the test conditions are altered. Therefore, to see whether the behavioural effects of ACTH could be counteracted by anxiolytics we tested the animals under 3 different conditions. A dose of 5 μg/100 g of ACTH was chosen as this was shown to be significant in Experiment 1 and produced less variable results than did the higher dose. The 2 drugs used were chronically administered chlordiazepoxide (5 mg kg^{-1}) and acutely administered ethanol (0·4 g kg^{-1}) since these had previously been shown to have

anxiolytic actions in the social interaction test (File, & others, 1976).

Methods

Animals. The animals were housed and handled as in Experiment 1. The rats given chronic chlordiazepoxide (CDP) received 5 mg kg^{-1} daily, for 5 days before the social interaction test, and their controls received 5 days of deionized water injections.

Apparatus. As in Experiment 1, but 2 levels of illuminance were used: 338 and 23·5 scotopic lux, for the high and low light levels respectively.

Drugs. Chlordiazepoxide hydrochloride (Roche Products Ltd) was dissolved in deionized water to a concentration of 2·5 mg ml^{-1}. Spectroscopically pure absolute ethanol (BDH Chemicals Ltd) was dissolved in deionized water to give a concentration of 0·16 g ml^{-1} (20% v/v). ACTH was dissolved as in Experiment 1.

Procedure. 252 rats were randomly allocated to the three test conditions: low light familiar, low light unfamiliar and high light unfamiliar. They were also randomly allocated to the 7 drug groups: (1) controls (½ received saline & ½ had water) (2) ACTH (5 μg/100 g), (3) ethanol (0·4 g kg^{-1}), (4) ethanol + ACTH, (5) CDP (5 mg kg^{-1} for 5 days), (6) chronic CDP + ACTH and 7) water (for 5 days). All rats received the same injection volume. Six pairs of rats were tested in each drug group and in each test condition.

For two days before the social interaction test the rats allocated to the low light familiar test condition were placed singly in the test box for 10 min. Rats allocated to the unfamiliar test conditions were placed in the test room, in the appropriate light level, for 10 min sessions, but remained in their home cages.

The animals were tested and scored for social interaction as described in Experiment 1. The rats were given intraperitoneal injections of CDP and ethanol 30 min before the test and of ACTH 3 min before the test.

Results

There was no difference in the scores of the control animals given an injection of water and those given saline, therefore the data were combined. The data were subjected to two-way analyses of variance in which the drug treatment was one factor and the test condition was the other. An anxiolytic action would be revealed by a significant drug × test condition interaction.

The control animals showed a decrease in social interaction across the test conditions (see Fig. 2) and ACTH significantly reduced social interaction in all the conditions ($F(1,30) = 58{\cdot}4$, $P < 0{\cdot}001$). Ethanol resulted in a significant drug × test condition interaction ($F(2,30) = 4{\cdot}2$, $P < 0{\cdot}025$), i.e. had a significant anxiolytic effect. Ethanol in combination with ACTH produced scores that did not differ significantly from the controls in any way.

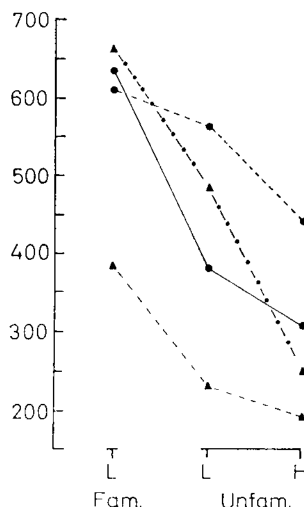

FIG. 2. Effects of ACTH and ethanol on active social interaction in rats. ●——● Controls, ▲- - -▲ ACTH (5 μg/100 g), ●- - -● ethanol (0·4 g kg^{-1}), ▲- - -●- - -●- - -▲ ethanol + ACTH. Ordinate: Active social interaction (s). Abscissa: Light level, L-Low, H-High, Fam.—familiar, Unfam.—Unfamiliar.

One possibility is that the decrease in active social interaction caused by ACTH is due to an anxiogenic action of the hormone. However, sedative drugs also decrease active interaction (File & others, 1976) and it is therefore necessary to consider other measures to distinguish between anxiogenic and sedative actions. In neither Expt 1 nor in this experiment was the level of motor activity reduced in ACTH-injected rats, and thus there was no evidence from these scores that ACTH had a sedative action. Sedative drugs also produce a dose-related increase in passive contact (File & others, 1976), whereas ACTH produced virtually no passive contact in any of the test conditions and the scores did not differ from those of the control animals.

Chronic CDP also produced an anxiolytic effect, i.e. a significant drug × test condition interaction

($F(2,30) = 4{\cdot}7$, $P < 0{\cdot}02$), see Fig. 3. The ACTH scores have not been replotted on Fig. 3, but the combination of ACTH and chronic CDP is shown. This combination did not produce a significant drug effect when compared with controls ($F(1,30) = 2{\cdot}6$), but the drug × test condition interaction was still significant ($F(2,30) = 4{\cdot}6$, $P < 0{\cdot}02$).

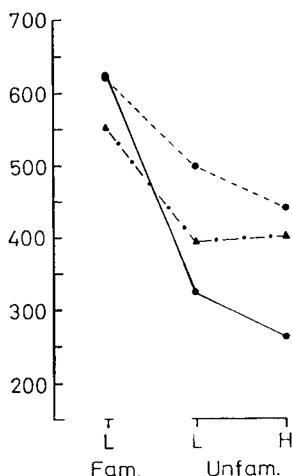

FIG. 3. Effects of chronic chlordiazepoxide and ACTH on active social interaction in rats. ●——● Controls, ●- - -● CDP (5 mg kg^{-1} for 5 days), ▲- - -●- - -● - - -▲ CDP + ACTH. Ordinate: Active social interaction (s). Abscissa: Light level, L—Low, H—High, Fam.—Familiar, Unfam.—Unfamiliar.

EXPERIMENT 3—EFFECTS OF ANXIOLYTIC DRUGS AND OF ACTH ON THE CONCENTRATIONS OF 5-HT AND OF 5-HIAA IN VARIOUS REGIONS OF RAT BRAIN

In this experiment we investigated the effects of ACTH and of anxiolytic drugs on 5-HT and 5-HIAA in various brain regions. Acute CDP was included for comparison with chronic CDP because with acute administration this drug had a sedative action in the social interaction test (File & others, 1976) and thus the behavioural effects differ from those seen with chronic administration of the drug.

Methods

Animals. The animals were housed and handled before use as described in Experiment 1.

Drugs. These were as in Experiments 1 & 2.

Procedure. All animals were decapitated between 10.00 h and 10.30 h, the brains rapidly removed, chilled in methanol (which had been pre-cooled to approximately −60° by an acetone/solid CO_2 mixture) and stored on solid CO_2 until required for assay. Brain regions were dissected according to the scheme of Glowinski & Iversen (1966):— hypothalamus, midbrain, hippocampus and cerebral cortex. The tissue samples were weighed and homogenized in 3·5 ml ice-cold n-butanol. 5-Hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations were determined fluorimetrically in individual brain regions by the method of Curzon & Green (1970), which is sensitive enough to enable both 5-HT and 5-HIAA to be measured in small regions in a single rat brain.

The following groups of animals were used: (1) water injected, animals killed 30 min after injection; (2) CDP (acute, 5 mg kg^{-1}), animals killed 30 min after injection; (3) CDP (chronic, 5 mg kg^{-1} per day for 5 days), animals killed 30 min after the last injection; (4) ethanol (0·4 g kg^{-1}), animals killed 30 min after injection; (5) saline, animals killed 10 min later; (6) ACTH injected, animals killed 10 min after injection.

Results

The effects of drug and ACTH administration on the concentrations of 5-HT and 5-HIAA are shown in Tables 1 & 2. Unfortunately there is no method available for measuring the true turnover rate of 5-HT (Persson & Waldeck, 1971) and thus we have restricted ourselves to considering changes in turnover (i.e. whether there has been an increase or a decrease). One way of expressing changes in neurotransmitter function is to determine the ratio of 5-HIAA: 5-HT, which gives an indication of 5-HT turnover, an increase indicating an increased turnover, and decrease the converse.

Table 1. *5-HT concentrations (nmol g^{-1}) in various brain regions after drug and hormone administration.*

Treatment	Hypothalamus	Midbrain	Hippocampus	Cerebral cortex
Water (n = 8)	11·9 ± 0·9	5·4 ± 0·3	8·4 ± 0·7	4·5 ± 0·3
Acute CDP (n = 6)	10·2 ± 1·2	4·9 ± 0·1	7·0 ± 0·8	4·1 ± 0·5
Chronic CDP (n = 8)	**13.7 ± 1·4	*7·0 ± 0·6	9·1 ± 0·8	****6·2 ± 0·4
Acute ethanol (n = 12)	11·9 ± 0·9	5·4 ± 0·3	7·4 ± 0·6	4·1 ± 0·4
Saline (n = 6)	8·2 ± 0·5	4·5 ± 0·3	6·0 ± 0·4	3·2 ± 0·5
ACTH (n = 6)	7·2 ± 0·7	3·9 ± 0·3	5·4 ± 0·2	2·5 ± 0·1

Each value is the mean (± s.e.m.).
Significantly different from corresponding controls (two-tailed *t* tests): * $P < 0{\cdot}025$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}005$; **** $P < 0{\cdot}001$.

Table 2. *5-HIAA concentrations (nmol g⁻¹) in various brain regions after drug and hormone administration.*

Treatment	Hypo-thalamus	Mid-brain	Hippo-campus	Cerebral cortex
Water (n = 8)	1·9 ± 0·15	1·1 ± 0·2	0·9 ± 0·01	1·0 ± 0·2
Acute CDP (n = 6)	2·0 ± 0·04	1·4 ± 0·2	1·0 ± 0·3	0·8 ± 0·2
Chronic CDP (n = 7)	†1·0 ± 0·02	0·8 ± 0·1	0·9 ± 0·1	0·6 ± 0·1
Acute ethanol (n = 13)	1·4 ± 0·1	†0·7 ± 0·05	1·1 ± 0·1	†0·6 ± 0·05
Saline (n = 6)	2·3 ± 0·4	1·6 ± 0·1	1·1 ± 0·02	1·1 ± 0·3
ACTH (n = 6)	*3·4 ± 0·07	***3·3 ± 0·04	†2·2 ± 0·3	1·2 ± 0·1

Each value is the mean (± s.e.m.).
Significantly different from corresponding controls (two-tailed *t*-tests): † $P < 0·05$; * $P < 0·025$; *** $P < 0·005$.

A difference between acute and chronic treatment with CDP was observed. After chronic treatment there was a significant decrease in the value of the 5-HIAA:5-HT ratio in the hypothalamus, midbrain and cerebral cortex. In contrast, a single injection of the drug had no effect on it (Table 3).

Acute treatment with ethanol caused a significant decrease in the ratio in the midbrain and hypothalamus. In contrast to the results with anxiolytic drugs, ACTH caused a significant increase in the ratio in the hypothalamus and midbrain (Table 3).

GENERAL DISCUSSION

The results of Experiment 3 are in general agreement with those of other investigators who have reported that benzodiazepines increase endogenous concentrations of 5-HT in the brain (Chase, Katz & Kopin, 1970) and reduce 5-HT turnover in the cerebral cortex (Lidbrink, Corrodi & others, 1973)

Table 3. *Ratio of 5-HIAA: 5-HT in various brain regions after drug and ACTH injection.*

Treatment	Hypo-thalamus	Mid-brain	Hippo-campus	Cerebral cortex
Water (n = 8)	0·16 ±0·02	0·2 ±0·05	0·1 ±0·01	0·2 ±0·04
Acute CDP (n = 6)	0·2 ±0·03	0·29 ±0·03	0·1 ±0·005	0·2 ±0·03
Chronic CDP (n = 7)	†0·07 ±0·005	†0·1 ±0·01	0·9 ±0·02	****0·09 ±0·01
Acute ethanol (n 12)	0·1 ±0·02	†0·1 ±0·01	0·15 ±0·01	†0·15 ±0·001
Saline (n = 6)	0·3 ±0·01	0·35 ±0·01	0·2 ±0·01	0·3 ±0·02
ACTH (n = 6)	†0·4 ±0·02	****0·8 ±0·04	0·4 ±0·04	0·4 ±0·05

Each value is the mean (±s.e.m.). The numbers of animals used is shown in parentheses.
Significantly different from corresponding controls (two-tailed *t*-tests):—† $P < 0·05$; **** $P < 0·001$.

and in the mid-brain-hindbrain regions (Stein & others, 1973). However, a direct comparison between findings is difficult as these authors used different methods for the assessment of 5-HT turnover and/or different benzodiazepine derivatives.

A variety of stressful stimuli have been shown to increase the concentration of 5-HIAA in the brain, without altering the concentration of 5-HT (Bliss, Thatcher & Ailion, 1972) and to increase 5-HT turnover (Glowinski, 1970; Morgan, Rudeen & Pfeil, 1975). In the present experiments a similar result was obtained with ACTH treatment. Our animals were killed 10 min after injection of ACTH, and although it is unlikely within this time interval that a marked increase in plasma corticosterone concentrations would have occurred (Hodges & Sadow, 1967; Hodges & Vellucci, 1975), it must be remembered that corticosterone itself can either increase or decrease tryptaminergic neuron function, depending on the dose (Curzon & Green, 1968; Telegdy & Vermes, 1975; Kovacs, Telegdy & Lissak, 1976).

To what extent could the behavioural results we obtained with ACTH be attributed to the action of endogenously released corticosterone? The effect of ACTH was maximal if the test was conducted in the period 3–13 min after injection, during which time it is unlikely that there was a marked increase in plasma corticosterone (Hodges & Sadow, 1967; Hodges & Vellucci, 1975). There would be higher corticosterone concentrations during the test period 15–25 min after injection, but at this time the effect on social interaction was less marked. It is possible that corticosterone has an anxiolytic effect and thus was counteracting the effects of ACTH and, indeed, corticosterone and ACTH have been reported to have opposite neural and behavioural effects (Steiner, Ruf & Akert, 1969; DeWied, 1974). We feel it is unlikely that corticosterone is anxiogenic because when the rats were tested 30 min after intraperitoneal injection of ACTH, when corticosterone concentrations would be high, but ACTH concentrations minimal, there was no significant effect on social interaction. However, the role of corticosterone in anxiety, and in particular the possibility that it could be anxiolytic, needs further investigation.

ACTH decreased social interaction in all the test conditions and this effect cannot be attributed to any sedative effect of the hormone. The results would be consistent with the interpretation that ACTH has an anxiogenic action, especially as its behavioural effects can be counteracted by anxiolytic drugs.

Since ACTH and anxiolytic drugs had opposite effects on 5-HT turnover, it is possible that one of the critical biochemical events in anxiety is the neural action of ACTH on 5-HT pathways in the midbrain and hypothalamus. By reducing turnover of 5-HT in these areas anxiolytic drugs could counteract the effects of ACTH. This does not, of course, exclude the possibility that other neurotransmitters also play an essential role in anxiety.

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