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# Influence of adrenocorticotrophic hormone on the behaviour in the swim test of rats treated chronically with desipramine

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**Abstract**—Chronic desipramine (DMI) administration induced a dose-dependent reduction in the immobility time of the swim test in rats. A combined treatment of ACTH (50 iu $kg^{-1}$ s.c.) and DMI (5 or 10 mg $kg^{-1}$ i.p.) for 7 days potentiated the anti-immobility effect of DMI. ACTH 4–10, a fragment peptide with little corticotrophic activity, mimicked ACTH-induced potentiation. No stimulating effect on locomotor activity was observed following seven daily co-administrations of ACTH or ACTH 4–10 and DMI (10 mg $kg^{-1}$). This behavioural evidence indicates that ACTH potentiation involves a central mechanism and demonstrates a functional interaction between ACTH and DMI at the behavioural level.

The clinical effects of various antidepressant treatments usually require several weeks to develop (Oswald et al 1972; Prange & Sulser 1972). Chronic, but not acute, administration of different classes of antidepressant drug, reduces cAMP accumulation activated by noradrenaline (Vetulani & Sulser 1975; Vetulani et al 1976; Mishra et al 1980) and the number of β-adrenoceptors in the cerebral cortex (Banerjee et al 1977; Bergstrom & Kellar 1979; Peroutka & Snyder 1980), with a time-course similar to the onset of clinical therapeutic effects. Thus, down-regulation of β-adrenoceptors is taken as a screening device to predict effective clinical antidepressant responses.

On the other hand, various peptide hormones have been described as capable of influencing the number and function of brain transmitter receptors (O'Donohue & Dorsa 1982; Kendall et al 1982; DeWied & Jolles 1982; Duman et al 1985), in addition to their known endocrine effects (O'Donohue & Dorsa 1982). In the frontal cortex, the co-administration of ACTH and DMI has been reported to induce accelerated onset of reduction in the number of β-adrenoceptors and a concomitant decline in the potency of noradrenaline to stimulate cAMP accumulation (Kendall et al 1982). However, no data are available about whether these neurochemical findings have consequences at the behavioural level.

Therefore, behavioural studies were undertaken to test this possibility. After chronic DMI and ACTH co-administration rats were tested in the 'behavioural despair' test, a model of experimental depression (Porsolt et al 1978).

## Materials and methods

*Animals.* Male Wistar rats, 250–320 g, were housed under a 12 h light-dark schedule with free access to water and food.

*Drugs.* Desipramine HCl (DMI) and purified ACTH were obtained from Montpellier Lab. (Buenos Aires, Argentina) and ELEA Lab. (Buenos Aires, Argentina), respectively. ACTH 4–10 was a gift from Organon (Oss, Holland). DMI and ACTH were dissolved in 0·9% NaCl (saline) and ACTH 4–10 was prepared in a zinc phosphate vehicle (DeWied & Bohus 1966) to prolong its half-life.

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*Porsolt test.* Rats were individually induced to swim inside a vertical plexiglas cylinder (height: 40 cm; diameter: 18 cm) containing 20 cm of water at 25 °C. After the rats had been in the water for 15 min, they were removed and allowed to dry for 30 min at 30 °C. Seven days later, and 1 h after the last drug treatment, the animals were replaced in the cylinder and immobility was tested during 5 min (Borsini et al 1984). A rat was judged to be immobile when it remained floating passively in the water, in an upright position, making only small movements to maintain its head just above the water.

*Measurement of locomotor activity.* A square open-field (60 × 60 × 60 cm) was used. Its floor was painted grey and divided into 15 × 15 cm squares by black lines. The test was performed under white light in a quiet room. Animals were taken from their cages and placed in the central square of the open-field. Locomotion was measured by the number of squares entered with all four paws and scored during a 5 min period. Testing was always performed 1 h after the last dose of drug treatment.

*Experimental design.* In chronic experiments, DMI was administered at a dose of 5, 10 or 20 mg $kg^{-1}$ once daily, i.p., for seven days. ACTH was injected s.c. once daily at a dose of 50 iu $kg^{-1}$ for a week. The first dose of DMI or ACTH was injected at the end of the drying period (i.e. 30 min after removal from the water). The last dose was given 1 h before the beginning of the immobility test. For ACTH-DMI combined treatment, the peptide was co-administered for 7 days with DMI (5, 10 or 20 mg $kg^{-1}$). The first dose of DMI was injected at the end of the drying period and the peptide was given 5 min later. This sequence was repeated every day for seven consecutive days. On the 7th day, 1 h after the last treatment, the duration of immobility was measured. An additional set of experiments was carried out with a group of animals that received DMI (10 mg $kg^{-1}$ $day^{-1}$) and ACTH 4–10 (20 μg/animal $day^{-1}$ s.c.) for 7 days, as described above. In acute experiments with ACTH or ACTH 4–10, rats were treated with DMI(10 mg $kg^{-1}$) or saline during 7 days, and received a single dose of ACTH (50 iu $kg^{-1}$) or ACTH 4–10 (20 μg/animal) 5 min after the last DMI dose. All injections were made between 1000h 1200h. The groups of control animals received saline (0·9% NaCl).

*Statistical analysis.* Multiple comparisons were made with an analysis of variance (ANOVA). Post-hoc comparisons were performed using Tukey's test. All data are expressed as mean ± s.e.m.

## Results

Fig. 1 shows that seven daily DMI injections induced a dose-dependent reduction of the duration of immobility time ($F(3,72) = 93{\cdot}6$, $P < 0{\cdot}001$). Chronic ACTH administration alone did not affect the total immobility duration. However, the combined administration of ACTH and DMI (5 or 10 mg $kg^{-1}$ $day^{-1}$) potentiated the anti-immobility effect of DMI

($F(1,72) = 15{\cdot}698$, $P < 0{\cdot}001$; interaction ACTH/DMI: $F(3,72) = 6{\cdot}88$, $P < 0{\cdot}001$). To test whether the effect of ACTH was mediated by adrenal hormones, the peptide fragment ACTH 4–10 was injected with DMI (10 mg kg$^{-1}$). This fragment, although centrally active, has little effect on the release of steroid hormones (DeWied 1969). Like ACTH, the centrally active peptide induced a higher anti-immobility response when administered in combination with DMI (Table 1: $F(5,48) = 65{\cdot}72$, $P < 0{\cdot}001$). Chronic ACTH 4–10 administered alone did not alter the immobility period as compared with saline-treated animals (Table 1).

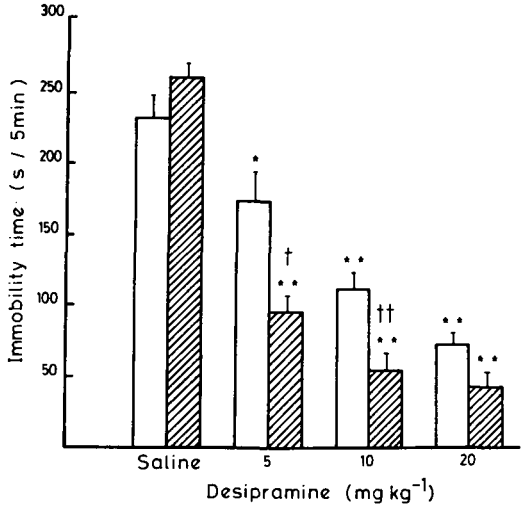


FIG. 1. Effect of the chronic combined treatment with ACTH and DMI on the duration of immobility. Each value is the mean ± s.e.m. for 10 animals. Statistical analyses were carried out by two-way ANOVA and post-hoc comparison by Tukey's test. $^{*}P < 0{\cdot}05$ compared with saline. $^{**}P < 0{\cdot}01$ compared with saline. $^{+}P < 0{\cdot}01$ compared with DMI (5 mg kg$^{-1}$). $^{++}P < 0{\cdot}05$ compared with DMI (10 mg kg$^{-1}$). Key: □ saline; ■ ACTH (50 iu kg$^{-1}$).

Table 1. Effect of ACTH 4–10 on the reduction of immobility caused by a 7-day treatment with DMI. ACTH 4–10 (20 μg/rat, s.c.) and DMI (10 mg kg$^{-1}$, i.p.) were administered as described under 'Materials and methods'. Values are means ± s.e.m.

| Treatment | N | Duration of immobility (min) |
|---|---|---|
| Saline | 10 | 233·2 ± 16·4 |
| Saline + ACTH 4–10 | 7 | 222·7 ± 11·3 |
| Saline + DMI | 10 | 113·5 ± 11·3* |
| DMI + ACTH 4–10 | 7 | 49·6 ± 12·1** |

Statistical analysis were carried out by one-way ANOVA and post-hoc comparison by Tukey's test.
* $P < 0{\cdot}01$ compared with saline.
** $P < 0{\cdot}01$ compared with saline + DMI.

Acute ACTH or ACTH 4–10 injection was incapable of modifying the response of saline—or chronic DMI (10 mg kg$^{-1}$)—pretreated animals (saline + ACTH = 234·6 ± 14·25; DMI + ACTH = 126 ± 26·77, saline + ACTH 4–10 = 236·6 ± 19·5; DMI + ACTH 4–10 = 125·8 ± 24·3).

The administration of ACTH, ACTH 4–10 or DMI alone did not modify locomotor basal activity after seven days of treatment (Table 2). Similarly, no stimulant effect was observed in the open-field test following the co-administration of ACTH or ACTH 4–10 and DMI (Table 2).

Table 2. The effect of the combined treatment with ACTH (50 iu kg$^{-1}$) or ACTH 4–10 (20 μg/animal day$^{-1}$) and DMI (10 mg kg$^{-1}$ day$^{-1}$) for 7 days on an open field activity (number of quadrants entered) during 5 min tests. Details are described under 'Materials and methods'. Values are mean ± s.e.m.

| Treatment | n | Open field activity (no. of squares) |
|---|---|---|
| Saline | 8 | 42·37 ± 6·72 |
| Saline + DMI | 7 | 34·86 ± 3·11 |
| Saline + ACTH | 7 | 40·2 ± 3·04 |
| DMI + ACTH | 6 | 33·3 ± 6·50 |
| Saline + ACTH 4–10 | 7 | 49·8 ± 5·7 |
| DMI + ACTH 4–10 | 7 | 51·0 ± 12·7 |

## Discussion

A prolonged treatment with DMI induced a dose-dependent reduction of immobility time in the swim test, as has been previously reported (Porsolt et al 1978; Borsini et al 1984; Kawashima et al 1986). This anti-immobility effect of DMI was potentiated by purified ACTH, at a dose which by itself had no effect.

Neither single nor combined ACTH/DMI chronic treatments modified motor activity scores in the open field test. Thus, it seems unlikely that a stimulating motor effect would be involved in the potentiation that ACTH exerts on the anti-immobility effect of DMI.

Biochemical studies supporting an ACTH-DMI interaction have shown that ACTH at the dose used is capable of accelerating, but not of magnifying, the down regulation of β-adrenoceptors evoked by chronic DMI (Kendall et al 1982). Although it can not be fully discarded, the DMI effect in the swim test seems unlikely to be mediated by down-regulation of β-adrenoceptors (Borsini et al 1984; Duncan et al 1985, 1986). Thus, a subsensitivity of these adrenoceptor sites is probably not required for the ACTH potentiation observed in our experiments.

Several reports have proposed a central dopaminergic mediation in the reduction of immobility time following chronic DMI (Borsini et al 1981, 1984, 1985). Considering that behavioural and biochemical evidence has shown an influence of ACTH on dopaminergic parameters (Wiegnat et al 1977; Iuvone et al 1978; Versteeg et al 1986), the potentiation we observed could be mediated through a central dopaminergic mechanism. However, further investigations are necessary to test this.

Because ACTH administration increases the release of steroid hormones, it is probable that the response to this peptide is mediated by adrenocorticoids. This possibility was examined by probing the effect of ACTH 4–10, an ACTH fragment that is centrally active but does not stimulate the release of adrenal hormones (DeWied 1969). ACTH 4–10 chronically combined with DMI significantly reduced the duration of immobility in the swim test, as did ACTH. This suggests that a central mechanism is involved in the potentiation induced by ACTH.

In summary, the evidence presented demonstrates a functional interaction of ACTH and DMI at the behavioural level, as tested by the forced swim test.

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# The guinea-pig trachea *O*-methylating system is more effective in modulating $\beta_2$- than $\beta_1$-adrenoceptor-mediated responses to isoprenaline

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**Abstract**—Assuming that responses of the guinea-pig trachea to isoprenaline in the presence of atenolol (10 μmol $L^{-1}$) are exclusively, or at least predominantly, $\beta_2$-adrenoceptor mediated and that responses to isoprenaline in the presence of ICI 118,551 (erythro-DL-1(7-methylindan-4-yloxyl)-3-isopropylaminobutan-2-ol) (1 nmol $L^{-1}$) are exclusively, or at least predominantly $\beta_1$-adrenoceptor mediated, the influence of inhibition of COMT by U-0521 (dehydroxy-2-methyl propiophenone) (50 μmol $L^{-1}$) has been compared in both conditions. U-0521 enhanced $\beta_2$-adrenoceptor mediated responses to isoprenaline 3·3-fold, while those mediated by $\beta_1$-adrenoceptors were enhanced only 2·2-fold. It is concluded that in guinea-pig trachea COMT activity is functionally more effective in modulating responses which are mediated by $\beta_2$-adrenoceptors than responses mediated by $\beta_1$-adrenoceptors.

In vascular tissue and for a given dose, the concentration of noradrenaline and adrenaline available for their α-adrenoceptor effects is mainly governed by uptake into the sympathetic nerve terminals, while *O*-methylation is the main factor determining the concentration of those agonists available for the β-effect (Guimarães 1982).

On the other hand, it has been suggested that the $\beta_1$-adrenoceptor subtype responds primarily to neurotransmitter and is therefore innervated, whereas the $\beta_2$-adrenoceptor is non-innervated and responsive to circulating catecholamines (Ariëns & Simonis 1976; Russel & Moran 1980; Bryan et al 1981; Broadley et al 1984).

The present investigation was undertaken to compare the influence of the *O*-methylating system on responses mediated by $\beta_1$- and $\beta_2$-adrenoceptors in the guinea-pig trachea—a tissue where both $\beta_1$- and $\beta_2$-adrenoceptors exist (Furchgott 1976; O'Donnell & Wanstall 1979).

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## Materials and methods

Guinea-pigs, 320–460 g, were killed by a blow on the head and bled. The trachea was removed and placed in bubbled Krebs-Henseleit solution, cleaned of excess tissue and cut spirally (Constantine 1965). Each strip was approximately halved and each half suspended in a 25 mL organ bath containing Krebs solution with added EDTA (27 μmol $L^{-1}$) and ascorbic acid (56 μmol $L^{-1}$) saturated with 95% $O_2$ + 5% $CO_2$. Preparations were contracted with 0·3 μmol $L^{-1}$ carbachol and relaxation responses were recorded by means of an isotonic myograph transducer, model MK II ser. 175, and amplifier, model CA 200, on a Physiograph DMP 4A (Narco Byosystems). The tension used was of about 1 g.

Cumulative concentration-response curves to isoprenaline were determined by the method of stepwise cumulative addition of the agonist. The concentration of the agonist in the bathing solution was increased 3-fold at each step, with each addition being made only after the response to the previous addition had