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Changes in the behavioural response to a TRH analogue following chronic amitriptyline treatment and repeated electroconvulsive shock in the rat

G.W. Bennett, A.R. Green*, Celia Lighton[1] & C.A. Marsden

Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH and M.R.C. Clinical Pharmacology Unit*, Radcliffe Infirmary, Woodstock Road, Oxford

1 The arousal elicited in rats by injection into the nucelus accumbens of the thyrotrophin-releasing hormone analogue CG 3509 (orotyl-histidyl-prolineamide) was used to assess the responsiveness to thyrotrophin-releasing hormone following repeated treatment with amitriptyline or electroconvulsive shock.

2 Fourteen day administration of amitriptyline (15 mg kg^{-1} i.p. twice daily) reduced the behavioural response to bilateral intra-accumbens injection of CG 3509 ($2 \times 2.5\,\mu g$). CG 3509-induced hyperactivity, recovery from pentobarbitone-induced anaesthesia and the reversal of both pentobarbitone-induced hypothermia and decreased respiration, were all significantly reduced compared to either the response of the animals prior to amitriptyline administration or that observed in rats following chronic saline administration.

3 Repeated administration of electroconvulsive shock (5 shocks over 10 days) significantly increased CG 3509-induced hyperactivity and the degree of reversal of pentobarbitone-induced hypothermia and respiratory depression following CG 3509 administration.

4 The results demonstrate that chronic antidepressant treatments alter the central functional responsiveness to thyrotrophin-releasing hormone. These changes are discussed with respect to the effects of antidepressant treatments on 5-hydroxytryptamine receptors and possible thyrotrophin-releasing hormone – aminergic interactions.

Introduction

There is evidence for a close relationship between the neuropeptide, thyrotrophin releasing hormone (TRH) and 5-hydroxytryptaminergic neurones in selective regions of the CNS. Fourteen days following administration of the 5-hydroxytryptamine (5-HT) neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), the reduction of endogenous levels and *ex vivo* release of TRH in the ventral spinal cord and nucleus accumbens of the rat suggests the co-existence of TRH and 5-HT in neurones within these two regions (Gilbert *et al.*, 1982; Lighton *et al.*, 1984a). The selective reduction in TRH content of the nucleus accumbens and the *ex vivo* release of TRH from the ventral spinal cord after administration of *p*-chlorophenylalanine (PCPA) further indicates a TRH/5-HT interaction at these sites (Lighton *et al.*, 1984a). The functional significance of such a relationship is as yet uncertain. However, evidence suggests that in these brain regions, TRH may induce 5-HT release (Crespi *et al.*, 1984), and in the spinal cord may act directly on the 5-HT receptor (Barbeau & Bedard, 1981).

A considerable amount of research has implicated 5-HT in both the aetiology and treatment of depressive illness. Moreover, Prange *et al.* (1972) reported the antidepressant potential of TRH, though subsequent clinical trials have not confirmed this (see Morley, 1979). More recently a number of the stable analogues of TRH have proved active in empirical tests used for new antidepressant drugs (Metcalf, 1983) and an antidepressive effect of TRH and its analogues has been shown in an animal model of depression (Ogawa *et al.*, 1984). In addition, endogenous levels of TRH in the rat nucleus accumbens and ventral spinal cord are selectively affected by chronic antidepressnst treat-

[1]Present address: Department of Pharmaceutical Chemistry, Mallot Hall, University of Kansas, Lawrence, Kansas 66045, U.S.A.

ment, amitriptyline administration causing an increase, and repeated electroconvulsive shock resulting in a decrease in peptide content (Lighton *et al.*, 1985).

Administration of TRH, and TRH analogues, results in (a) hyperactivity of the conscious animal (Miyamoto & Nagawa, 1977; Sharp *et al.*, 1984a) and (b) antagonism of the narcosis induced by a number of centrally depressant drugs (Breese *et al.*, 1975; Sharp *et al.*, 1984b). The potency of TRH analogues to elicit TSH release and to compete for [^{3}H]-TRH binding sites (Dettmar *et al.*, 1983) suggests they have a direct action on the TRH-binding site. In the present study we have used the above two models of TRH analogue-induced arousal to determine the effects of chronic antidepressant drug treatment and electroconvulsive shock on the responsiveness of neurones in the nucleus accumbens to TRH.

Methods

Implantation of guide cannulae into the nucleus accumbens

Stainless steel guide cannulae (23 gauge) were stereotaxically implanted bilaterally into the nucleus accumbens of male Wistar rats (280 ± 10 g) under pentobarbitone anaesthesia (60 mg kg^{-1}, i.p.). Stereotaxic co-ordinates for the nucleus accumbens were measured from bregma and the dura surface as A/P +3.2; L/R ±1.1; V −7.2 (Pellegrino & Cushman, 1967). Guide cannulae were positioned 1.0 mm above the target nuclei and kept patent by removable stainless steel stylets. Following recovery, animals were maintained on a 12 h light-dark cycle (lights on 8 h 00 min–20 h 00 min) with food and water *ad libitum*. Seven days were allowed before further experimentation.

Chronic administration of amitriptyline

Cannulated rats were administered amitriptyline (15 mg kg^{-1}, i.p. twice daily, Roche Products Ltd) for 14 days. Control animals received an equivalent volume of 0.9% NaCl solution w/v. All injections were made at 9 h 00 min and 19 h 00 min.

Repeated administration of electroconvulsive shock

Cannulated rats were lightly anaesthetized with halothane and given electroconvulsive shock (ECS) (125 V, 50 Hz, sinusoidal) for 1 s through ear-clip electrodes from a small animal electroplexy unit (Theratronics, Guernsey). Five shocks were administered over 10 days (days 1, 3, 5, 8 and 10). Control animals were repeatedly anaesthetized, had electrodes attached, but no current was passed (sham-shocked).

CG 3509-induced hyperactivity

The measurement of CG 3509-induced hyperactivity was performed using an Actimat doppler shift radar animal activity meter (Marsden & King, 1979). Following removal of the cannulae stylets, animals were acclimatized for 1 h to individual housing in the Actimat enclosure. Animals were then removed and restrained by hand during bilateral injection of orotyl-histidyl-prolineamide (CG 3509; 2 × 2.5 μg in 0.5 μl saline, Grunenthal GmBH, Aachen). Injection was made over a 2 min period, using a 31 gauge needle (measured to extend 0.1 mm beyond the guide cannulae and into the target region) attached to a microinfusion syringe pump (Harvard Apparatus Ltd). The injection needle was removed after a further 30 s and the animals returned to the observation box. Total activity counts were monitored in 15 min periods, for a total of 120 min. Twenty-four hours later saline (0.9%, 2 × 0.5 μl) was injected bilaterally and activity similarly monitored.

CG 3509-induced reduction of pentobarbitone sleep time

The effect of microinjections of CG 3509 or saline on pentobarbitone-induced sleep time was measured on a further group of cannulated animals. All experiments were performed in a soundproof, temperature-regulated room (22 ± 1°C). Sleeping time was measured as the duration of loss of righting reflex (LRR) following sodium pentobarbitone (35 mg kg^{-1}, i.p.). Animals were placed on their sides and 20 min after LRR, bilateral injections of CG 3509 (2 × 2.5 μg) were made into the nucleus accumbens. Recovery of righting reflex (RRR) was judged as the time when the animal was able to right its upper torso three times within 60 s. Rat core temperature was monitored at 10 min intervals with a thermistor probe, inserted 3–4 cm into the rectum and connected to a digital read-out thermometer. Respiration rate was measured each 10 min by counting the number of thoracic respiratory movements over a 60 s period (breaths per min). Twenty-four h later, the effects of bilateral intra-accumbens injection of 0.9% saline (2 × 0.5 μl) on the duration of sleep time, rectal temperature and respiratory rate, induced by pentobarbitone administration, was measured. Preliminary groups of animals were tested for analepsis in response to repeated administration of intra-accumbens CG 3509 and the responses compared to either initial or final saline injections were determined.

Experimental protocol

The experimental protocol used to determine the effect of chronic amitriptyline on CG 3509-induced hyperac-

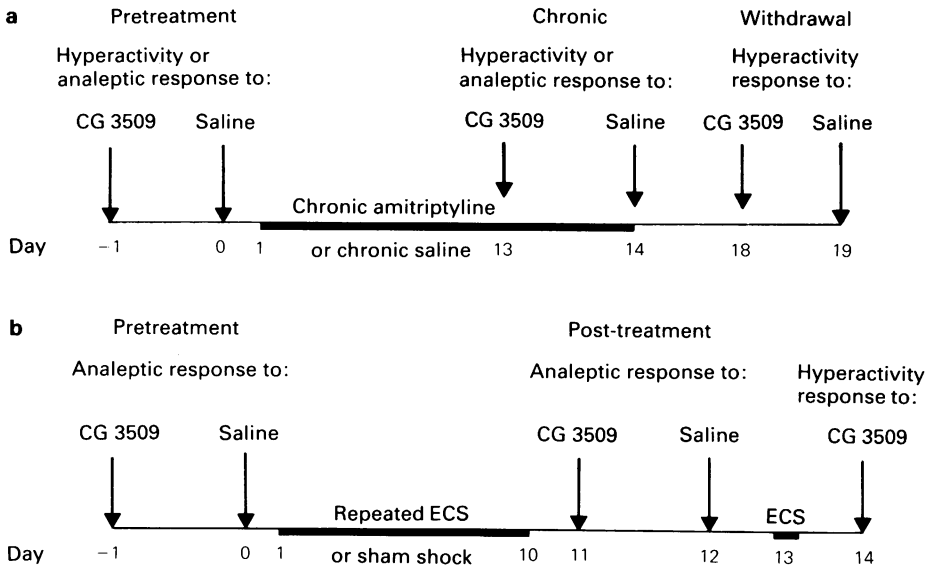

Figure 1 Experimental protocols used to determine the effect of chronic amitriptyline (15 mg kg^{-1}, i.p twice daily) treatment (a) and repeated electroconvulsive shock (ECS) (b) on CG 3509-induced hyperactivity and reduced pentobarbitone sleeping time.

tivity or analepsis is shown in Figure 1a.

Subsequent to recovery from surgical intervention, cannulated animals, allocated to receive amitriptyline, were measured for either bilateral intra-accumbens CG 3509 (2 × 2.5 μg)-induced hyperactivity or analepsis (day − 1), and 24 h later (day 0) for their corresponding response to intra-accumbens injection of saline (pretreatment response).

All animals were then chronically treated with amitriptyline (15 mg kg^{-1}, i.p. twice daily) or an equivalent volume of saline (days 1–14). On day 13 of chronic amitriptyline administration, the same animals, initially tested for either CG 3509-induced hyperactivity or analepsis, were again assessed. Twenty-four hours later, the effect of intra-accumbens saline was determined (chronic response). Four days after the end of treatment with amitriptyline the response of the animals to CG 3509 was tested again (withdrawal response).

The experimental protocol used to determine the effect of repeated ECS on CG 3509-induced hyperactivity and analepsis is shown in Figure 1b. A group of cannulated animals was measured for its analeptic response to bilateral intra-accumbens injection of CG 3509 (2 × 2.5 μg) and saline (days − 1 and 0 respectively) (pretreatment response). Animals were not determined for CG 3509-induced hyperactivity at this stage. Animals were then administered repeated electroconvulsive shock or repeated sham shock (days 1–10). Twenty-four hours after the final electroconvulsive shock, animals were again tested for CG 3509-induced analepsis (day 11). After a further 24 h, the analeptic response to intra-accumbens saline was determined (post-treatment response). Subsequent to the analeptic tests, the administration of repeated ECS was resumed, and animals were administered a further shock (day 13). On day 14 of the study, conscious animals were monitored for activity following bilateral intra-accumbens injection of CG 3509 (2 × 2.5 g) (Figure 1b). This protocol can be justified by the observation that the monoamine-modulated behavioural responses are maximally enhanced after treatment over 10 days and further intermittent shocks maintained this enhancement (Costain *et al.*, 1979).

Histological verification of central injection sites

The animals were killed with sodium pentobarbitone (i.p.) and pontamine sky blue (1.0% w/v) injected into the nucleus accumbens to determine the injection sites in frozen sections. All injection sites were located in the medial nucleus accumbens and were concentrated within an area ±0.4 mm of the chosen stereotaxic coordinates.

Data analysis

Parameters of CG 3509-induced behavioural arousal were compared by Student's *t* test, at all stages of

antidepressant treatment.

The effect of chronic antidepressant treatment on CG 3509-induced reduction of pentobarbitone sleep time, was determined from the ratio of the pentobarbitone sleep time following intra-accumbens saline: pentobarbitone sleep time following intra-accumbens CG 3509 (Kendall & Stuart, 1977).

Results

Effect of chronic amitriptyline treatment on CG 3509-induced hyperactivity

(a) *Pretreatment response to CG 3509* After a 60 min habituation period in the Actimat enclosure, the bilateral injection of saline into the nucleus accumbens, resulted in 40 min of mild exploratory behaviour (Figure 2). For the remainder of the 120 min post-injection period, total activity counts monitored by the Actimat continued to decline and animals showed few signs of arousal, sleeping for long intervals in the corner of the cage (Figure 2). Bilateral intra-accumbens injection of CG 3509 ($2 \times 2.5\,\mu g$) produced marked changes in behaviour consisting of excessive sniffing, licking, grooming, rearing, jerking of the head and whole-body shaking with infrequent movement across the cage. Total activity counts were highest in the 15 min immediately following CG 3509 administration (Figure 2) and were significantly higher than those following central injection of saline throughout the 120 min post-injection period (Figure 2). Comparisons of total activity counts in the 120 min post-injection period showed that CG 3509 ($2.5\,\mu g$ bilaterally) significantly increased behavioural activity compared with saline injected control values (Figure 3). In this study, as found previously (Sharp *et al.*, 1984a), the effect of repeated administration of intra-accumbens CG 3509 over several days was shown not to alter significantly the behavioural response to subsequent saline injections when compared with initial saline responses.

No significant difference was observed in the pretreatment response to either saline or CG 3509 between animals allocated to receive either chronic amitriptyline or saline control injections (Figure 3).

(b) *Response to CG 3509 following either chronic saline or amitriptyline* Chronic saline administration did not significantly alter activity compared to the pretreatment response following central injection of either saline or CG 3509 (Figure 3). However, activity following central injection of saline to rats treated chronically with amitriptyline was significantly increased ($P < 0.05$) compared to the response prior to amitriptyline treatment, although this activity did not differ from that observed following chronic saline

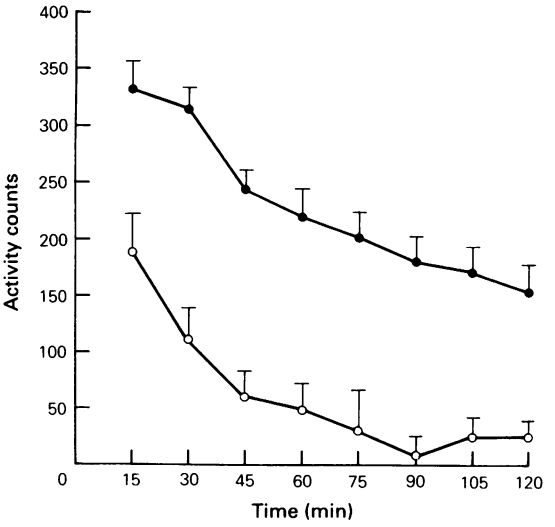

Figure 2 Effect of bilateral injection of CG 3509 into the nucleus accumbens on total activity counts. Saline (○) or CG 3509 ($2 \times 2.5\,\mu g$) (●) were injected ($1\,\mu l$) and activity recorded for 120 min. Results are expressed as mean activity, with vertical lines showing s.e.mean, of $n = 7$ or 9.

(Figure 3). Behavioural hyperactivity following CG 3509 was seen in rats treated with amitriptyline but the hyperactivity was significantly less than that measured either prior to antidepressant treatment or following chronic saline administration (Figure 3).

(c) *Withdrawal response to CG 3509* Four days following withdrawl from chronic amitriptyline, the behavioural activity induced by intra-accumbens injection of CG 3509, was still significantly reduced compared to both the pretreatment response and that in chronic saline treated animals (Figure 3).

Effect of chronic amitriptyline treatment on CG 3509-induced reduction in pentobarbitone sleep time

(a) *Pretreatment response to CG 3509* As found previously (Sharp *et al.*, 1984b), the analeptic effect of repeated administrations of intra-accumbens CG 3509 over several days was shown to produce similar responses in comparison with either initial or final saline responses. Bilateral injection of CG 3509 ($2 \times 2.5\,\mu g$) into the nucleus accumbens halved the duration of pentobarbitone sleep time compared with saline injected control values (Figure 4). No significant difference was measured in the response to either saline or CG 3509 ($2.5\,\mu g$ bilaterally) between the groups of animals allocated to receive chronic saline (controls) or amitriptyline (figure 4).

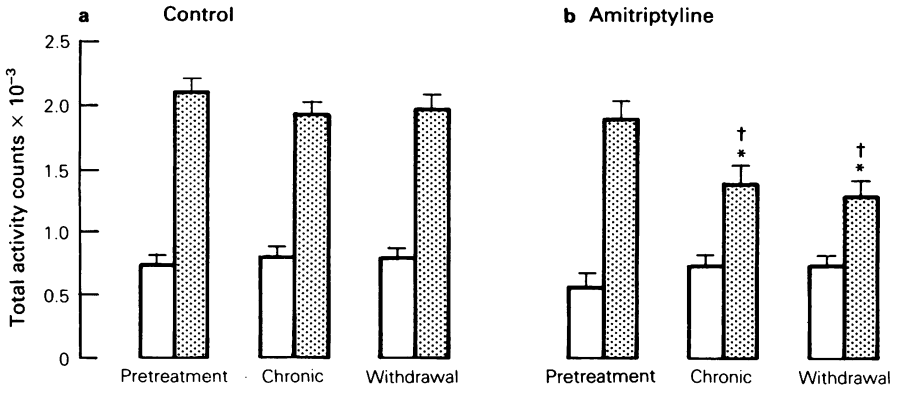

Figure 3 Effect of chronic (14 days) amitriptyline (15 mg kg^{-1} i.p., twice daily) on CG 3509-induced hyperactivity. CG 3509 (2 × 2.5 μg) (stippled columns) or saline (open columns) was bilaterally injected (1 μl) into the nucleus accumbens and activity monitored for 120 min post-injection. The injections were made prior to treatment with amitriptyline (pretreatment), during treatment (chronic) or 4 days after the end of amitriptyline treatment (withdrawal). Results are given as the total activity counts, with vertical bars showing s.e.mean, recorded during the 120 min test period in (a) control (n = 7) and (b) amitriptyline-treated (n = 9) rats. CG 3509 significantly ($P < 0.01$) increased activity compared to intra-accumbens saline at all three test times (pretreatment, chronic and withdrawal). Activity induced by CG 3509 was significantly less at the chronic and withdrawal test times compared to pretreatment. *$P < 0.01$ cf. intra-group and †$P < 0.05$ cf. inter-group comparison.

(b) *Response to CG 3509 following either chronic saline or amitriptyline* Chronic saline treatment did not significantly alter pentobarbitone sleep time in response to either central saline or CG 3509 injection (Figure 4). However, pentobarbitone-induced sleeping time was significantly increased following chronic amitriptyline, being more than double that of the pretreatment response group and of the chronic saline treated animals (Figure 4). Bilateral intra-accumbens injection of CG 3509 (2 × 2.5 μg), significantly shortened the duration of pentobarbitone sleep time in amitriptyline treated animals (Figure 4) but the reduction (−34%) was less than that recorded before chronic amitriptyline treatment and animals slept longer than those given chronic saline (Figure 4).

Effect of chronic amitriptyline on CG 3509-induced reversal of pentobarbitone-induced hypothermia

(a) *Pretreatment response to CG 3509* Administration of sodium pentobarbitone (35 mg kg^{-1}, i.p.), followed by intra-accumbens injection of saline decreased rat rectal temperature by 0.4 ± 1°C. This decrease was not observed in rats given CG 3509 (2.5 μg bilaterally). Rats allocated to receive chronic amitriptyline or chronic saline showed no difference in their response to either saline or CG 3509.

(b) *Response to CG 3509 following either chronic saline or amitriptyline* Rectal temperature measured at LRR was significantly lower after 14 days of amitriptyline treatment than that prior to amitriptyline administration or in animals given chronic saline (Table 1). During the 60 min after LRR there was a significantly greater decrease in temperature in the amitriptyline

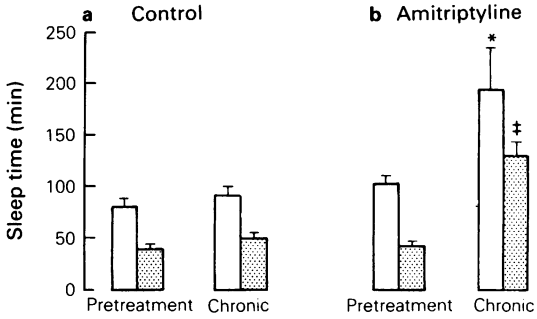

Figure 4 Effect of (b) chronic (14 days) amitriptyline (15 mg kg^{-1} i.p., twice daily) or (a) saline (control) on CG 3509-induced reduction in pentobarbitone (35 mg kg^{-1} i.p.) sleeping time. The columns represent mean sleep time, with vertical bars showing s.e.mean, (n = 7) following either bilateral intra-accumbens injection of saline (open columns) or CG 3509 (2 × 2.5 μg) (stippled columns). CG 3509 significantly decreased pentobarbitone sleeping time in both pretreated and amitriptyline treated rats ($P < 0.01$). Amitriptyline treatment significantly increased pentobarbitone-induced sleep and decreased the CG 3509 reduction in sleep time. *$P < 0.001$, ‡$P < 0.05$ (both inter- and intra-group comparison).

Table 1 Effect of chronic amitriptyline treatment on the CG 3509 reversal of pentobarbitone-induced hypothermia

	Temperature (°C)	
	Pre-CG 3509 (LRR)	*Post-CG 3509 (change in 20 min)*
Pretreatment (14)	37.5 ± 0.2	+0.07 ± 0.07
Chronic saline (7)	37.0 ± 0.1	+0.10 ± 0.04
Chronic amitriptyline (7)	36.4 ± 0.2*	−0.46 ± 0.14*

Temperature was measured at loss of righting reflex (LRR) following administration of pentobarbitone (35 mg kg^{-1}), 20 min post LRR when CG 3509 (2 × 2.5 μg) was injected into the nucleus accumbens and 20 min post-CG 3509 administration. Results are given as the value at LRR and the difference between the values when CG 3509 was injected and 20 min post-injection.
*$P < 0.05$ compared to pretreatment and chronic saline values.

treated animals (−1.1 ± 0.5°C) compared with the pretreatment response (−0.34 ± 0.1°C; $P < 0.05$).

Chronic amitriptyline prevented the reversal of pentobarbitone hypothermia caused by CG 3509 (2.5 μg bilaterally). A comparison of the change in rectal temperature in the 20 min immediately following CG 3509 injection showed that while in amitriptyline treated animals temperature continued to decrease there was an increase in both pretreated and chronic saline treated animals (Table 1).

Effect of chronic amitriptyline on CG 3509-induced reversal of pentobarbitone-induced respiratory depression

(a) *Pretreatment response to CG 3509* Injection of pentobarbitone (35 mg kg^{-1}), resulted in an initial rapid fall in respiration rate in the 10 min immediately following LRR. After this, respiration rate steadily increased until at RRR it was equal to or slightly exceeded that at LRR. Intra-accumbens injection of CG 3509 (2.5 μg bilaterally) caused a rapid increase in respiration rate, that was significantly higher than that following saline administration ($P < 0.05$).

(b) *Response to CG 3509 following either saline or amitriptyline* Chronic saline administration did not alter the respiratory response to intra-accumbens saline and CG 3509 compared to the pretreatment responses. However, chronic amitriptyline markedly prolonged the respiratory response in anaesthetized animals receiving intracerebral saline so that 60 min after LRR, and until RRR, the respiration rate of amitriptyline treated animals was significantly lower than that measured at pretreatment ($P < 0.02$).

Intra-accumbens injection of CG 3509 (2.5 ± g bilaterally), to amitriptyline treated animals, significantly increased the respiration rate. However, the increase was not significant until 10 min after the time recorded at pretreatment. During the 20 min following CG 3509 (2.5 μg bilaterally), the increase in respiration rate in the amitriptyline treated animals was + 9.4 ± 5.7 breaths min^{-1} compared to values in the pretreated and chronic saline treated animals of + 22.8 ± 3.5 and + 16.3 ± 3.8 breaths min^{-1}, respectively.

Effect of repeated ECS and CG 3509-induced hyperactivity

Post-treatment response to CG 3509 Twenty-four hour following repeated ECS, animals were monitored for hyperactivity induced by bilateral intra-accumbens injection of CG 3509 (2 × 2.5 μg). Total activity counts accumulated in the 120 min following analogue injection, showed that repeated ECS enhanced hyperactivity induced by CG 3509 (+11%; $P < 0.05$) (Figure 5b). A comparison of total activity accounts accumulated in 15 min intervals, showed that animals receiving ECS, maintained a higher level of CG 3509-induced activity throughout the 120 min study period (Figure 5a).

Effect of repeated ECS on CG 3509-induced reduction of pentobarbitone sleep time

Post-treatment response to CG 3509 Twenty-four hour following repeated ECS or sham shock, the duration of pentobarbitone sleep time, subsequent to saline injection (66.9 ± 5.0 min), was not significantly different from that determined at pretreatment. Similarly, the reduction in sleep time, induced by CG 3509, both in animals receiving repeated ECS (−31%, $P < 0.01$) or in sham shocked animals (−32%; $P < 0.01$), was not significantly different from the pretreatment values.

Effect of repeated ECS on CG 3509-induced reversal of pentobarbitone hypothermia

Post-treatment response to CG 3509 Twenty-four hours after repeated ECS the mean rectal temperature

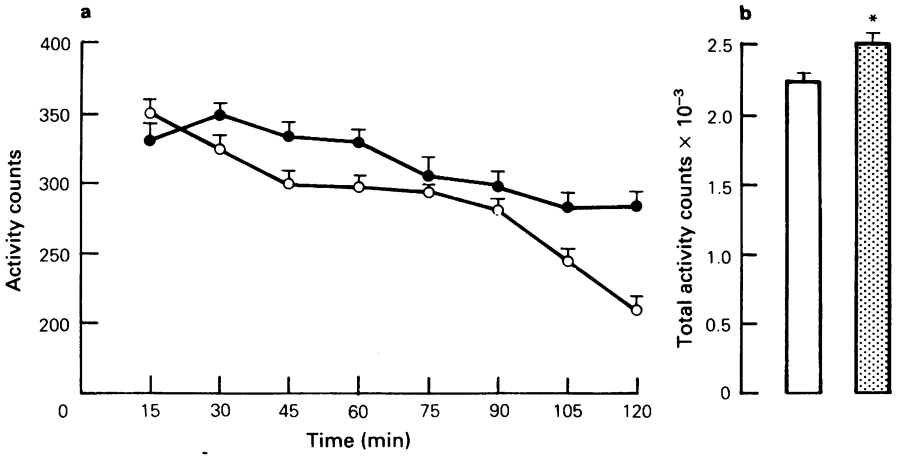

Figure 5 Effect of repeated electroconvulsive shock (ECS) on CG 3509-induced hyperactivity. CG 3509 (2 × 2.5 μg) was administered bilaterally into the nucleus accumbens of rats given either repeated ECS (a (●) and b, stippled columns; $n = 5$) or sham shocks (a (○) and b, open columns; $n = 8$) and activity monitored every 15 min for 120 min. Results are expressed in (a) as counts every 15 min, with vertical lines showing s.e.mean, and (b) as total activity during 120 min test period. $^*P < 0.05$ compared to sham shocked group (unpaired t test).

measued at LRR was significantly higher than that measured at pretreatment or following sham shock treatment (Table 2). In the 60 min after LRR there was a significantly greater reduction in temperature following repeated ECS (-1.45 ± 0.20°C) than in pretreated animals (-0.63 ± 0.11°C; $P < 0.02$) but not compared to the sham shocked group (-1.12 ± 0.15°C).

In ECS treated animals, bilateral administration of CG 3509 (2 × 2.5 μg) produced a significantly greater reversal of the hypothermia induced by pentobarbitone than that measured at pretreatment or in sham shocked animals (Table 2).

Effect of repeated ECS on CG 3509-induced reversal of pentobarbitone respiratory depression

Post-treatment response to CG 3509 There was no significant difference in the respiratory response to intra-accumbens saline or to CG 3509 between pretreatment or sham shocked rats.

Repeated ECS enhanced the respiratory response to CG 3509. In the 20 min immediately following CG 3509 injection, the increase in respiration rate of ECS treated animals ($+29.5 \pm 4.0$ breaths min^{-1}) was higher than that recorded at pretreatment ($+18.1 \pm 5.7$ breaths min^{-1}) and significantly higher

Table 2 Effect of repeated electroconvulsive shock (ECS) on the CG 3509 reversal of pentobarbitone-induced hypothermia

	Temperature (°C)	
	Pre-CG 3509 (LRR)	*Post-CG 3509 (change in 20 min)*
Pretreatment (16)	37.0 ± 0.3	$+0.30 \pm 0.09$
Sham shock (8)	37.2 ± 0.2	$+0.11 \pm 0.01$
ECS (8)	38.1 ± 0.2*†	$+0.74 \pm 0.15$*‡

Temperature was measured at loss of righting reflex (LRR) after administration of pentobarbitone (35 mg kg^{-1}), 20 min post LRR when CG 3509 (2 × 2.5 μg) was injected into the nucleus accumbens and 20 min post-CG 3509 administration. Results are given as the value at LRR and the difference between the values when CG 3509 was injected and 20 min post-injection. Details of the ECS/sham shock protocol are given in Figure 1b.
†$P < 0.01$; ‡$P < 0.001$, compared to sham shock. *$P < 0.02$ compared to pretreatment.

($P < 0.05$) than that in sham shock animals ($+15.6 \pm 4.6$ breaths min^{-1}).

Discussion

This study confirms previous findings that the stable TRH analogue, CG 3509 induces behavioural arousal and analepsis when injected into the nucleus accumbens (Heal *et al.*, 1981; Sharp *et al.*, 1984a,b). In addition, it shows that chronic administration of amitriptyline significantly reduced the level of behavioural hyperactivity induced by the injection of CG 3509 into the nucleus accumbens. Comparable with the CG 3509-iduced changes in the conscious animals, chronic amitriptyline treatment also reduced the analeptic response to CG 3509 measured in the pentobarbitone-anaesthetized rat. While chronic administration of amitriptyline by itself significantly increased the duration of pentobarbitone sleep time, intra-accumbens injection of CG 3509 into amitriptyline-treated animals still reduced the duration of anaesthesia, although the animals slept significantly longer than before drug treatment. The decrease in CG 3509-induced hyperactivity and the suggested reduction in analeptic response to CG 3509 would indicate that chronic amitriptyline treatment decreases TRH responsiveness which may possibly reflect a subsensitivity of TRH binding sites in the nucleus accumbens.

Within the brain, TRH has been suggested to function as an endogenous ergotroph (Metcalf & Dettmar, 1981). The apparent subsensitivity of TRH binding sites following amitriptyline administration may result in a reduced response to the endogenous peptide, so delaying the arousal normally mediated by endogenous TRH and increasing pentobarbitone sleep time following chronic amitriptyline treatment. Hirsch (1983) has demonstrated that barbiturate analogues compete directly for the TRH binding site and have suggested such an interaction may regulate the duration of pentobarbitone-induced narcosis. However, the mechanisms by which barbiturates depress synaptic transmission, and thereby induce anaesthesia, appear multiple and it is unlikely that their effects on TRH binding fully explain their anaesthetic properties. A selective, amitriptyline-induced, subsensitivity of the competitive TRH-barbiturate binding site ('arousal site'), however, may result in the increased duration of narcosis measured after amitriptyline.

The reduction in the duration of pentobarbitone anaesthesia by CG 3509 was accompanied by a reversal of the associated hypothermia and respiratory depression. Interestingly, chronic amitriptyline also attenuated both the hyperthermic and respiratory responses to CG 3509. Since analeptic doses of either TRH or its analogues cause a rapid increase in respiration rate, it has been suggested that peptide activation of a central respiratory mechanism is a primary factor in TRH-induced arousal (Carino *et al.*, 1976). Indeed following chronic amitriptyline treatment, the prolonged duration of narcosis was accompanied by a persistently low respiration rate, which only displayed a gradual increase in the 20 min immediately prior to arousal. However, because of the prolonged duration of sleep time measured following amitriptyline, and the accompanying hypothermia and respiratory depression, it is difficult to say whether the suggested decrease in analeptic response to CG 3509 was a result of the subsensitivity of TRH binding sites or because the animal was under a 'deeper' level of anaesthesia. Lin (1978) has observed that treatment with the antidepressant chlorimipramine lowers body temperature by reducing metabolic rate. The decrease in body temperature, respiration rate and increase in duration of anaesthesia caused by amitriptyline, could indicate a similar decrease in metabolic rate.

In contrast to chronic amitriptyline administration, repeated ECS enhanced the hyperactivity induced by CG 3509. Similarly, following repeated ECS intra-accumbens injection of CG 3509 resulted in a greater reversal of pentobarbitone-induced hypothermia and respiratory depression. However, repeated ECS altered neither the reduction in pentobarbitone sleep time after CG 3509 nor the duration of sleeping time in the absence of CG 3509 from that measured at pretreatment, the latter confirming the finding of Evans *et al.* (1976). This apparent dissociation between CG 3509-induced increase in respiration and analepsis may relate to the analeptic dose-response curve of TRH (Sharp *et al.*, 1984b). These authors showed that the dose-response curves of CG 3509, when injected into the nucleus accumbens, indicate a plateau in the analeptic response, which probably reflects the specific number of saturable TRH binding sites in the accumbens (Sharif & Burt, 1983). The lack of effect of repeated ECS on CG 3509 reduction in pentobarbitone sleeping time may therefore reflect the high dose of analogue used and the near maximal analeptic response elicited. The alternative suggestion is that the increase in respiration induced by CG 3509 is one of a number of responses which jointly initiate arousal.

In earlier studies we investigated the effect of chronic antidepressant treatment and repeated ECS on the levels and *ex vivo* release of TRH in selected areas of rat brain and spinal cord (Lighton *et al.*, 1984b; 1985). The results indicated that chronic amitriptyline administration increased and repeated ECS decreased the synthesis and *ex vivo* release of TRH-like immunoreactivity in the nucleus accumbens. The present results demonstrate that within this

brain region chronic amitriptyline treatment attenuates, whilst repeated ECS enhances, the behavioural response to direct injection of a TRH analogue. When the biochemical and behavioural responses are considered together, the results suggest that in the nucleus accumbens, amitriptyline stimulates the synthesis and release of TRH-LI, which in turn down-regulates the sensitivity of TRH-binding sites. If this is so, the increase in level of hyperactivity induced by CG 3509 following repeated ECS may be a consequence of the up-regulation of TRH binding sites in the nucleus accumbens, resulting from decreased synthesis and release of TRH-LI. That changes in the endogenous levels of TRH-LI may regulate TRH binding sites in the CNS is indicated from the results of Ogawa *et al.* (1983). These authors demonstrated that chronic administration of TRH (i.p.) produced a significant reduction in the number of TRH binding sites in the hippocampus, hypothalamus and cerebral cortex of the rodent brain. Furthermore, the increase in the number of spinal TRH-binding sites in rats depleted of endogenous spinal TRH-LI is compatible with TRH modulation of TRH receptor number (Sharif *et al.*, 1983).

Lesioning 5-HT neurones with 5,7-dihydroxytryptamine decreases TRH levels and *ex vivo* release in the nucleus accumbens and septum (Lighton *et al.*, 1984a) as well as in the ventral lumbar cord as previously reported (Gilbert *et al.*, 1982; Marsden *et al.*, 1982). Furthermore chronic treatment with an antidepressant with effects on both 5-HT uptake and 5-HT_2-receptors (Ögren *et al.*, 1979; Stolz *et al.*, 1983) increases TRH levels and *ex vivo* release in the nucleus accumbens (Lighton *et al.*, 1985). Several studies using both ligand binding and behavioural techniques have demonstrated reduced 5-HT_2-receptor sensitivity following chronic antidepressant treatment (Peroutka & Snyder, 1980; Kellar *et al.*, 1981a; Tang *et al.*, 1981; Ögren *et al.*, 1982; Stolz *et al.*, 1983; Fuxe *et al.*, 1983). In contrast, repeated ECS increases 5-HT_2-binding and enhances 5-HT_2-receptor mediated behaviour (Green *et al.*, 1977; Vetulan *et al.*, Green *et al.*, 1983) and increases TRH levels and *ex vivo* release in the nucleus accumbens (Lighton *et al.*, 1984b). In the present study we have shown that chronic amitriptyline decreases behavioural respones produced by the TRH analogue CG 3509 while chronic ECS enhances the same responses. Thus an antidepressant drug and ECS produce the same reciprocal changes in 5-HT and TRH receptors and function. Fuxe *et al.* (1983) have suggested that some of the adaptive changes in 5-HT receptors induced by chronic antidepressant treatment are mediated via modulatory cofactors with which they interact. A possible mechanism for these effects is that TRH acts directly on the postsynaptic 5-HT receptor to regulate binding to and responses elicited by the receptor, and recently Metz & Green (unpublished observation) have shown *in vitro* that under certain conditions TRH decreases 5-HT_2-ligand binding in the nucleus accumbens. Alternatively, TRH and 5-HT may interact indirectly via a pre- or post-synaptic mechanism. Interestingly, we have recently shown that 5,7-dihydroxytryptamine lesions prevent the amitriptyline-induced increase in TRH (Bennett *et al.*, 1985), suggesting that the antidepressant-induced changes in TRH levels and functional responsiveness are dependent on intact 5-HT neurones.

This study has measured hyperactivity induced by CG 3509, an effect mediated via dopamine systems (Miyamoto & Nagawa, 1977; Heal & Green, 1979; Sharp *et al.*, 1984a). The suggested decrease in TRH receptor responsiveness following amitriptyline treatment could result in decreased TRH receptor-mediated dopamine release (Heal & Green, 1979; Sharp *et al.*, 1982) and consequently the reduced hyperlocomotor response to CG 3509. Also, the reduction in pentobarbitone-induced narcosis, elicited by TRH and its analogues, may be mediated by cholinergic mechanisms (Brunello & Cheney, 1981; Yarbrough, 1983). Amitriptyline has potent anticholinergic properties (Hollister, 1978) and following withdrawl from chronic treatment, the number of striatal muscarinic binding sites is increased (Koide & Matsushita, 1981). Such antagonism by amitriptyline may result in the prolonged duration of anaesthesia induced by the drug in the present study and in the reduced analeptic effect of CG 3509. Furthermore, repeated ECS appears devoid of any effect on rat cortical muscarinic binding (Deakin *et al.*, 1981; Kellar *et al.*, 1981b), and no alteration in pentobarbitone-induced sleeping time was evident in the present study.

In summary, the results show that chronic amitriptyline treatment reduced the arousal effects of the TRH analogue CG 3509 while ECS enhanced these effects. It remains to be determined whether these alterations in TRH responsiveness relate to the antidepressant properties of amitriptyline and ECS and their effects on amine neurones.

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