Effect of chronic intra-accumbens administration of the TRH analogue CG3509 on histamine-induced behaviour in the rat

¹L.J. Bristow & ²G.W. Bennett

Department of Physiology & Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH

1 The present study has investigated the effect of chronic intra-accumbens administration of the thyrotrophin-releasing hormone (TRH) analogue, CG3509, on CG3509- and histamine-induced spontaneous motor activity and brain TRH-like immunoreactive (TRH-LI) levels in the rat.

2 Chronic intra-accumbens administration of CG3509 ($5 \times 5 \mu\text{g}$ over 3 days) induced: (a) a significant ($P < 0.05$) reduction in intra-accumbens CG3509 ($0.5 \mu\text{g}$)-induced hyperactivity, (b) reduced levels of TRH-LI in the nucleus accumbens but not other brain regions, (c) a marked increase (107%, $P < 0.01$) in histamine-induced non-locomotor hyperactivity.

3 The present results demonstrate that alteration of central TRH function following treatment with a TRH analogue enhances the effect of intra-accumbens histamine on behavioural hyperactivity, possibly via changes in H_1 receptors and suggest that the neuropeptide, TRH and histamine interact in behavioural arousal mechanisms in rat brain.

Introduction

Accumulating evidence supports a role for histamine as a neurotransmitter/neuromodulator in the CNS (see reviews Prell & Green, 1986; Pollard & Schwartz, 1987). Furthermore, recent studies (Bristow & Bennett, 1988a) suggest that the behavioural arousal mechanisms of this amine (Kalivas, 1982) may reside, in part, within the nucleus accumbens, since marked hyperactivity occurs in response to intra-accumbens administration and the histamine H_1 -receptors mediating this response have been identified (Bristow & Bennett, 1988a). Interestingly, histamine-induced hyperactivity shows many similarities to that previously described following intra-accumbens administration of the neuropeptide, thyrotrophin releasing hormone (TRH) (Heal & Green, 1979; Sharp *et al.*, 1984; Kalivas *et al.*, 1987). Similarly, specific TRH receptor binding sites are present within the nucleus accumbens (Taylor & Burt, 1982; Sharif & Burt, 1983; Rostene *et al.*, 1984) and TRH release from rat accumbens slices has been described (Bennett *et al.*, 1981). The present study has therefore investigated possible intra-accumbens interactions between TRH

and the histaminergic system in rats by use of behavioural techniques. Previous evidence indicates that histamine may interact with TRH in the accumbens, since histamine induces TRH release from synaptosomal preparations (Barracough *et al.*, 1983) and manipulation of endogenous brain histamine levels is associated with marked changes in accumbens levels of TRH (Bennett *et al.*, 1983).

In an attempt to counter the poor biological stability and short-lasting actions of TRH, several structural analogues have been produced by modifications to the N-terminal pyroGlu and C-terminal Pro-NH₂ residues of the parent peptide. Various neuropharmacological studies have shown that these analogues are more potent than TRH on account of their enhanced biological stability (Metcalf, 1983). Thus microinjection of TRH analogues into the nucleus accumbens induces a greater and longer-lasting hyperactivity response than that seen with TRH (Sharp *et al.*, 1984). Furthermore, chronic treatment with TRH analogues results in the development of a behavioural tolerance to the peptide (Simasko & Horita, 1985; Fone *et al.*, 1988), which may be a consequence of a down regulation of central TRH receptors (Ogawa *et al.*, 1983; Simasko & Horita, 1985). In the present study, rats were chronically treated with the analogue CG3509

¹ Present address: Merck, Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM23 2QR.

² Author for correspondence.

(orotyl-L-histidyl-L-prolineamide), as a method for manipulating central TRH receptor-mediated function, and the effects on the hyperactivity response induced by CG3509 and on histamine-induced behaviour were investigated. The effect of chronic TRH analogue treatment on the levels of TRH-like immunoreactivity (TRH-LI) in selected brain regions was also determined.

Methods

Bilateral cannulation of the nucleus accumbens

Male Wistar rats (300 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} , i.p.) and bilaterally implanted with stainless steel guide cannulae (23 gauge, 15 mm length) according to the atlas of Paxinos & Watson (1982); A/P + 1.7; L/R $\pm$ 1.4; V – 3 mm. A minimum of 7 days was allowed before the experiments were commenced, during this time cannulae were kept patent by a removable stainless steel stylet (31 gauge, 15 mm length).

Activity measurements

Drug-induced changes in activity were monitored using the Actimat doppler shift radar activity meter (Marsden & King, 1979), as described previously (Bristow & Bennett, 1988a). This system measures both the speed and duration of rat activity by directing a 10 W energy microwave beam (10.5 GHz) from a doppler radar module into an experimental enclosure ($60 \times 70 \times 65 \text{ cm}$) and analyses the small frequency changes of the reflected signals. Activity occurring in the frequency band of 0.4–4 Hz largely represents movements of the head and body without actual locomotion. Conversely, movements in the frequency band 4–100 Hz reflect high speed activity consisting largely of locomotion around the cage.

Individual rats were allowed an hour to acclimatize to the system after which they were removed and lightly restrained by hand. Drug injections into each cannula were administered over a 1 min period, the injection needle was removed after a further 30 s and animals were returned to the enclosure. A 1 min recovery period was then allowed before activity measurements commenced. Behaviour was monitored in 15 min intervals for 1 or 2 h and expressed as activity counts, with one count representing 1 s of activity. Activity counts occurring in both high and low frequency wavelengths are presented.

Experimental procedures

All experiments were carried out between 09 h 00 min and 12 h 00 min and rats were familiarised with the experimental environment on at least 2

separate occasions before behavioural responses were tested. All rats were initially tested for their behavioural responses following intra-accumbens injection of (a) 0.9% saline ($2 \times 1 \mu\text{l}$, day 1), (b) histamine ($10 \mu\text{g} \mu\text{l}^{-1}$, $2 \times 1 \mu\text{l}$, day 3) and CG3509 ($0.5 \mu\text{g} \mu\text{l}^{-1}$, $2 \times 0.5 \mu\text{l}$, day 6). Rats were then chronically treated twice daily (09 h 00 min and 18 h 00 min) with either 0.9% saline ($2 \times 0.5 \mu\text{l}$) or CG3509 ($5 \mu\text{g} \mu\text{l}^{-1}$, $2 \times 0.5 \mu\text{l}$), 5 bilateral intra-accumbens injections given over 3 days (days 8–10). The behavioural response to CG3509 ($0.5 \mu\text{g} \mu\text{l}^{-1}$, $2 \times 0.5 \mu\text{l}$) was then retested 24 h later (day 11). Similarly, histamine ($10 \mu\text{g} \mu\text{l}^{-1}$, $2 \times 1 \mu\text{l}$)-induced activity was retested on day 12.

Measurement of tissue TRH levels

On completion of activity measurements (day 12) rats were stunned and decapitated and the nucleus accumbens, septal nuclei, corpus striatum and hypothalamus dissected according to Craigies anatomical atlas (1963) (Lighton *et al.*, 1984). Tissue samples were extracted in 1 ml 90% methanol, the supernatants dried down in a Buchler vortex evaporator at 60°C and stored at -80°C for radioimmunoassay. The tissue pellets were retained for protein analysis by the method of Lowry *et al.* (1951).

TRH-like immunoreactivity was measured by the radioimmunoassay method of Lighton *et al.* (1984) using ethanol precipitation to separate bound from free peptide. The antiserum, raised in sheep at Nottingham by the method of Bassiri & Utiger (1972), was used at a working assay dilution of 1:20 K and shown to cross-react only with peptides containing both the N- and C-terminal residues of synthetic TRH. Assay sensitivity was 28 fmol per tube and the intra-assay and inter-assay coefficients of variation were 2.1% and 12% respectively.

Peptide recovery through the extraction, drying and assaying procedures was assessed by spiking bilateral tissue halves with 10 ng exogenous synthetic TRH. A mean tissue recovery of $61 \pm 6\%$ ($n = 4$) was calculated.

Statistical analysis

The effects of chronic treatment with CG3509 on the behavioural responses induced by CG3509 and histamine were analysed by use of Student's paired *t* test to compare pre- and post-treatment responses in the same group. Data presented as time courses were analysed by 2-way analysis of variance for repeated measures (BMDP statistical software) and *F* values, degrees of freedom (d.f.) and *P* values presented where appropriate. The hyperactivity response induced by various CG3509 doses (Figure 2) was analysed by one way analysis of variance using

Peritz's *f*-test for multiple comparisons (Harper, 1984). Tissue TRH levels measured in various brain regions from rats chronically treated with CG3509 or saline were analysed by Student's *t* tests.

Drugs

Histamine dihydrochloride (Sigma) and CG3509 (Grunenthal GmbH, Aachen) were dissolved in 0.9% saline solution for intra-accumbens injections.

Results

Effect of intra-accumbens administration of saline, histamine and CG3509 on rat activity

Bilateral microinjection of histamine ($10 \mu\text{g } \mu\text{l}^{-1}$) into the nucleus accumbens induced marked biphasic changes in behavioural activity as demonstrated previously (Bristow & Bennett, 1988a,b). Thus in the first 15 min rats showed a significant ($P < 0.01$) reduction in activity counts, compared to saline controls, followed by a marked hyperactivity response (Figure 1). Intra-accumbens administration of the TRH analogue CG3509 (0.01 – $1 \mu\text{g } \mu\text{l}^{-1}$, $2 \times 0.5 \mu\text{l}$) induced a marked dose-dependent increase in activity counts, without an initial hypoactivity phase (Figure 2), similar to that described previously (Sharp *et al.*, 1984). Furthermore, subdivision of activity counts into those representing low speed activity (i.e. small body movements, Figure 2b) and high speed activity (i.e. locomotion, Figure 2c) showed that an increase in activity occurred in both these wavelengths. However, changes in the low frequency band accounted for some 80% of the total activity changes recorded in 2 h.

Figure 1 Effect of intra-accumbens injection of saline ($2 \times 1 \mu\text{l}$) (open columns) or histamine ($10 \mu\text{g}$, $2 \times 1 \mu\text{l}$) (hatched columns) on activity counts accumulated from (a) 0–15 min and (b) 15–120 min. Columns represent mean activity counts and bars show s.e.mean ($n = 7$); data analysed by Student's paired *t* test. $**P < 0.01$ compared to saline controls.

Figure 2 Effect of intra-accumbens microinjection of saline ($2 \times 0.5 \mu\text{l}$) (hatched columns) or CG3509 (0.01 – $1 \mu\text{g } \mu\text{l}^{-1}$, $2 \times 0.5 \mu\text{l}$) (open columns) on (a) total activity counts, (b) low frequency counts and (c) high frequency counts accumulated from 0–120 min. Columns represent mean activity counts and bars show s.e.mean ($n = 7$). Data were analysed by one way analysis of variance using Peritz's *f* test for multiple comparisons. $**P < 0.01$ compared to saline controls.

Effect of chronic treatment with CG3509 on CG3509-induced behaviour

After pretreatment testing of the behavioural responses to histamine and CG3509, rats were

Figure 3 Effect of chronic treatment with CG3509 ($5 \mu\text{g} \mu\text{l}^{-1}$, $2 \times 0.5 \mu\text{l}$, $n = 7$) or saline ($2 \times 0.5 \mu\text{l}$, $n = 7$) on CG3509 ($0.5 \mu\text{g} \mu\text{l}^{-1}$, $2 \times 0.5 \mu\text{l}$)-induced hyperactivity responses measured before (hatched columns) and after (stippled columns) chronic treatment. Columns represent mean activity counts accumulated from 0–60 min postinjection for (a) total activity counts, (b) low frequency counts and (c) high frequency counts; bars show s.e.mean. Data for each group were analysed by Student's paired *t* test. * $P < 0.05$ compared to pretreatment CG3509 response.

chronically treated twice daily with either 0.9% saline ($2 \times 0.5 \mu\text{l}$) or CG3509 ($5 \mu\text{g} \mu\text{l}^{-1}$, $2 \times 0.5 \mu\text{l}$), five intra-accumbens injections being given over 3 days. The behavioural response to CG3509 was then

Figure 4 Effect of chronic treatment with CG3509 on histamine ($10 \mu\text{g} \mu\text{l}^{-1}$, $2 \times 1 \mu\text{l}$)-induced behavioural changes (a) Histamine-induced activity counts recorded for 15 min intervals measured before (hatched columns) and after (stippled columns) chronic treatment. Open columns show activity counts following intra-accumbens saline ($2 \times 1 \mu\text{l}$) injection. In (a) and (b) columns represent mean activity counts ($n = 7$) and bars show s.e.mean. (a) Data (i.e. pretreatment vs post-treatment response) were analysed by 2 way analysis of variance for repeated measures: $F_{\text{treatment}} = 19.76$, d.f. 1, 6, $P = 0.0044$; $F_{\text{time}} = 10.07$, d.f. 7, 42, $P < 0.0001$; $F_{\text{interaction}} = 2.93$, d.f. 7, 42, $P = 0.0136$. (b) Total, low frequency and high frequency activity counts accumulated from 15–120 min following histamine administration before (hatched columns) and after (stippled columns) chronic CG3509 treatment. Data were analysed by Student's paired *t* test. ** $P < 0.01$ compared to pretreatment histamine response.

retested 24 h later. Rats chronically treated with the TRH analogue showed a small but significant ($P < 0.05$) reduction (20%) in CG3509-induced hyperactivity (Figure 3). In contrast, chronic treatment with saline did not alter the hyperactivity response induced by CG3509. Subdivision of total activity counts into those occurring in low (Figure 3b) and high (Figure 3c) frequency wavelengths revealed a significant ($P < 0.05$) reduction in CG3509 induced hyperactivity in both wavelengths, of 18% and 30% respectively.

Effect of chronic treatment with CG3509 on histamine-induced behaviour

Histamine ($10 \mu\text{g} \mu\text{l}^{-1}$, $2 \times 1 \mu\text{l}$)-induced behaviour following intra-accumbens injection was retested 48 h after the final chronic treatment with either

saline ($2 \times 0.5 \mu\text{l}$) or CG3509 ($5 \mu\text{g} \mu\text{l}^{-1}$, $2 \times 0.5 \mu\text{l}$). Chronic TRH analogue administration resulted in a marked increase (107%, $P < 0.01$, Figure 4) in histamine-induced hyperactivity represented by activity counts accumulated from 15–120 min, while activity counts recorded in the first 15 min were not significantly altered, suggesting that histamine-induced hypoactivity was unaffected (Figure 4a). Furthermore, subdivision of the total activity counts into those occurring in low and high frequency wavelengths showed that the increase in histamine-induced hyperactivity resulted from a significant ($P < 0.01$) increase in low frequency activity i.e. small body movements (Figure 4b). In contrast, locomotor behaviour represented by counts occurring in the high frequency wavelengths was not significantly altered (Figure 4b). Chronic treatment with saline did not alter either histamine induced hypo- or hyperactivity (Figure 5).

Effect of chronic CG3509 treatment on brain tissue TRH levels

On completion of activity testing, rats were killed and TRH content measured in (a) nucleus accumbens, (b) septal nuclei, (c) corpus striatum and (d) hypothalamus. Rats treated with chronic intra-accumbens CG3509 ($5 \mu\text{g} \mu\text{l}^{-1}$, $2 \times 0.5 \mu\text{l}$) showed a significant ($P < 0.05$) reduction in TRH levels in the nucleus accumbens (Table 1). In contrast, TRH levels measured in the remaining brain regions were not significantly different in chronic saline- or CG3509-treated rats.

Discussion

Chronic treatment with the TRH analogue CG3509 (5 intra-accumbens injections over 3 days) resulted in

Table 1 Effect of chronic treatment with saline or CG3509 ($5 \times 5 \mu\text{g} \mu\text{l}^{-1}$) on regional brain levels of thyrotrophin-releasing hormone-like immunoreactivity (TRH-LI) in the rat

Brain region	TRH ($\text{pg} \mu\text{g}^{-1}$ protein)	
	Saline treated	CG3509 treated
Nucleus accumbens	1.1 ± 0.3 (6)	$0.4 \pm 0.1^{**}$ (7)
Septum	1.3 ± 0.1 (5)	1.1 ± 0.2 (6)
Corpus striatum	0.3 ± 0.2 (3)	0.3 ± 0.1 (6)
Hypothalamus	2.6 ± 0.1 (7)	2.5 ± 0.1 (7)

After completion of behavioural measurements rats were killed and brain tissue TRH-LI levels measured by radioimmunoassay. Results are expressed as the mean tissue TRH levels $\pm$ s.e.mean; n = number in parentheses. $^{**}P < 0.01$, Student's t test.

Figure 5 Effect of chronic treatment with saline on histamine ($10 \mu\text{g} \mu\text{l}^{-1}$, $2 \times 1 \mu\text{l}$)-induced behaviour. (a) Histamine-induced activity counts recorded in 15 min intervals were measured before (hatched columns) and after (stippled columns) chronic saline treatment. Open columns show activity counts following saline control injections. In (a) and (b) columns represent mean activity counts ($n = 7$) and bars show s.e.mean. (a) Data (pretreatment response vs posttreatment response) were analysed by 2-way analysis of variance for repeated measures: $F_{\text{treatment}} = 0.38$, d.f. 1, 6, $P = 0.56$; $F_{\text{time}} = 6.37$, d.f. 7, 42, $P = 0.098$. (b) Total, low frequency and high frequency counts measured from 15–120 min following histamine administration before (hatched columns) and after (stippled columns) CG3509 treatment. Data were analysed by Student's paired t test and were not significantly different compared to pretreatment response in each frequency range.

a significant ($P < 0.05$) reduction in CG3509-induced hyperactivity responses compared to those measured before treatment. In contrast, chronic saline injection did not alter the behavioural response induced by the analogue. These results are consistent with those of previous studies which demonstrated the development of a behavioural tolerance following treatment with TRH analogues (Simasko & Horita, 1985; Fone *et al.*, 1988). Furthermore, this reduced behavioural response may be a consequence of a down regulation of TRH receptors, since Simasko & Horita demonstrated a significant reduction in [^3H]-3MeHis²TRH binding to brain homogenates on the third day of chronic treatment with the TRH analogue MK771.

After the behavioural responses had been measured in the present study the rats were killed, the brains removed and the nucleus accumbens and adjacent regions assayed for TRH content by radioimmunoassay. In rats chronically treated with

the TRH analogue the levels of TRH in the nucleus accumbens were significantly reduced ($P < 0.05$) compared to control saline-treated animals. In contrast, the levels of TRH measured in the septum, striatum and hypothalamus were not altered following CG3509 treatment. It is unlikely that this reduction in TRH results from interference of CG3509 in the assay procedure since TRH analogues show negligible cross-reactivity with the TRH-antisera preparation (Lighton *et al.*, 1984). Furthermore, since the change in endogenous peptide was restricted to the nucleus accumbens, this may suggest that selective changes in TRH synthesis and/or release may occur in addition to the down regulation of TRH receptors, predicted by the development of a behavioural tolerance to CG3509.

In addition to the changes induced in central TRH function, chronic CG3509 treatment was also associated with a marked selective increase in histamine-induced hyperactivity, shown previously to be mediated by H_1 receptors (Bristow & Bennett, 1988a). Interestingly, subdivision of the activity analysis into the major non-locomotor and minor locomotor components showed that this potentiation in histamine-induced hyperactivity was largely an effect on non-locomotor behaviour i.e. head and forepaw movements, sniffing and grooming (Bristow & Bennett, 1988a). In contrast, the transitory histamine-induced hypoactivity response observed in previous studies (Bristow & Bennett, 1988a,b) was unaltered by treatment with the TRH analogue. This suggests that presynaptic events, i.e. histamine synthesis and release, may not be modulated by TRH, since previous evidence (Bristow & Bennett, 1988b) would suggest that the hypoactivity is mediated by H_3 autoreceptors which are intimately involved with these processes (Arrang *et al.*, 1983; 1987). The present results, then, suggest that TRH present within and released from (Bennett *et al.*, 1981) the accumbens specifically modulates the arousal action of histamine at postsynaptic H_1 receptors. That neuropeptides are able to modulate the behavioural effects induced by neurotransmitter substances in the nucleus accumbens has been demonstrated previously, e.g., neurotensin blocks, whereas substance P enhances, dopamine mediated hyperlocomotion (Kalivas *et al.*, 1984; Kalivas & Miller, 1984).

Various previous studies have shown that intra-accumbens injection of TRH or its more stable analogues induces a marked hyperactivity response in rats (Miyamoto & Nagawa, 1977; Heal & Green, 1979; Heal *et al.*, 1981; Sharp *et al.*, 1984; Miyamoto *et al.*, 1984; Kalivas *et al.*, 1987). This behaviour, like that following intra-accumbens histamine, consists of changes in several different behaviours including locomotion, sniffing, grooming, and rearing, but also

includes forepaw licking and chewing, wet dog shakes and straub tail (Heal *et al.*, 1981; Sharp *et al.*, 1984). While the stereotypy and locomotor behaviours apparently involve accumbens and other fore-brain mechanisms (Sharp *et al.*, 1984), recent evidence suggests that wet dog shakes, forepaw licking and tail elevation result from actions on spinal or rhombencephalon neurones (Fone *et al.*, 1987; Johnson *et al.*, 1989). Thus the latter behaviours, observed following intra-accumbens injection of TRH analogues, possibly result from diffusion to more caudal brain regions.

There is a large body of evidence suggesting that TRH-induced hyperlocomotion is mediated via an increase in mesolimbic dopamine release (Miyamoto & Nagawa, 1977; Heal & Green, 1979; Sharp *et al.*, 1984), but other neurotransmitter systems have also been implicated in the hyperactivity response induced by this peptide. In particular, evidence suggests the involvement of the noradrenergic system in both rats (Lin *et al.*, 1983; Miyamoto *et al.*, 1984) and mice (Heal *et al.*, 1987). The present results now indicate that the histaminergic pathway from the mammillary bodies to the accumbens (Pollard & Schwartz, 1987) is also associated with mechanisms of arousal and may be modulated by changes in TRH receptor function.

Although the present and previous studies support an interaction between histamine and TRH in arousal mechanisms, the precise nature of the interaction remains unclear. Although histamine stimulates release of TRH from sheep accumbens synaptosomal preparations through actions on H_2 receptors (Barraclough *et al.*, 1983), it is unlikely that histamine-induced hyperactivity responses in the rat are directly mediated through stimulation of TRH release, since the potent and selective H_2 agonist dimaprit did not induce any behavioural effects and the response to histamine was not attenuated following pretreatment with the H_2 antagonist SKF93479 (Bristow & Bennett, 1988a). Moreover, if TRH-induced hyperactivity is mediated by dopaminergic mechanisms (see above) and histamine-induced hyperactivity were mediated via an increase in TRH release, then pretreatment with dopamine antagonists would be expected to block the effects of histamine. However, preliminary studies indicate that pretreatment with sulpiride, at doses shown to be effective in blocking amphetamine-induced locomotion (Sharp *et al.*, 1986), have no effect on histamine-induced behaviour (unpublished observations). In contrast, the selective modulation of histamine H_1 receptor-mediated hyperactivity responses, observed in the present study, may suggest interactions at the level of the H_1 receptor itself and preliminary binding studies have demonstrated increases in H_1 receptor number following

presentation with CG3509 (Bristow & Bennett, 1988c). It thus remains to be determined whether chronic TRH analogue treatment induces any changes in H_1 receptor sensitivity and whether this relates to the changes occurring in the histamine H_1 receptor-mediated hyperactivity.

In summary, chronic intra-accumbens treatment with the TRH analogue CG3509 is associated not only with the development of a behavioural toler-

ance to the peptide, but also with a marked increase in histamine H_1 receptor-mediated hyperactivity. These results provide evidence suggesting that histamine and TRH are closely linked in arousal mechanisms associated with the nucleus accumbens.

We thank Professor L. Flohe, Grunenthal GMBH for the generous supply of CG3509. L.J.B. was an SERC/CASE student with SK&F.

References

- ARRANG, J.M., GARBARG, M. & SCHWARTZ, J.C. (1983). Autoinhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature*, **302**, 832–837.
- ARRANG, J.M., GARBARG, M. & SCHWARTZ, J.C. (1987). Autoinhibition of histamine synthesis mediated by presynaptic H_3 receptors. *Neuroscience*, **23**, 149–157.
- BARRACLOUGH, A., HODGKINSON, D.W. & BENNETT, G.W. (1983). Histamine induced release of TRH from isolated nerve endings (synaptosomes). In *Thyrotrophin Releasing Hormone*, ed. Griffiths, E.C. & Bennett, G.W., pp. 351–352. New York: Raven Press.
- BASSIRI, R.M. & UTIGER, R.D. (1972). The preparation and specificity of antibody to thyrotrophin releasing hormone. *Endocrinology*, **90**, 722–727.
- BENNETT, G.W., COOPER, S., EGLEN, A. & MARSDEN, C.A. (1983). The effect of N-acetylhistamine on regional TRH levels in rat brain and spinal cord. *Br. J. Pharmacol.*, **80**, 641P.
- BENNETT, G.W., MARSDEN, C.A., SHARP, T. & STOLTZ, J.F. (1981). Concomitant determination of endogenous release of dopamine, noradrenaline, 5-hydroxytryptamine and thyrotrophin releasing hormone (TRH) from rat brain slices and synaptosomes. In *Central Neurotransmitter Turnover*, ed. Pycock, C.J. & Taberner, P.V., pp. 183–189. London: Croom Helm Ltd.
- BRISTOW, L.J. & BENNETT, G.W. (1988a). Biphasic effects of intra-accumbens histamine administration on spontaneous motor activity in the rat; a role for central histamine receptors. *Br. J. Pharmacol.*, **95**, 1292–1302.
- BRISTOW, L.J. & BENNETT, G.W. (1988b). A role for histamine H_3 receptors in histamine induced hypo-activity in the rat. *Br. J. Pharmacol.*, **94**, 319P.
- BRISTOW, L.J. & BENNETT, G.W. (1988c). Behavioural and biochemical evidence for TRH modulation of histamine H_1 receptor function in rat brain. *Br. J. Pharmacol.*, **95**, 886P.
- CRAIGIES, J.R.M. (1963). *Neuroanatomy of the Rat*. ed. Zernon, W. New York: Academic Press.
- FONE, K.C.F., BENNETT, G.W. & MARSDEN, C.A. (1987). Involvement of catecholaminergic neurones and α -adrenoceptors in the wet dog shake and forepaw licking behaviour produced by the intrathecal injection of an analogue of thyrotrophin releasing hormone (CG3509). *Neuropharmacology*, **26**, 1147–1155.
- FONE, K.C.F., DIX, P., TOMLINSON, D.R., BENNETT, G.W. & MARSDEN, C.A. (1988). Spinal effects of chronic intra-theal administration of the thyrotrophin releasing hormone analogue CG3509 in rats. *Brain Res.*, **455**, 157–161.
- HARPER, J.F. (1984). Peritz's f-test: Basic program of a robust multiple comparison test for statistical analysis of all differences among means. *Comput. Biol. Med.*, **14**, 437–445.
- HEAL, D.J. & GREEN, A.R. (1979). Administration of thyrotrophin releasing hormone (TRH) to rats releases dopamine in the n. accumbens but not n. caudatus. *Neuropharmacology*, **18**, 23–31.
- HEAL, D.J., SABBAGH, A., YODIM, M.B.H. & GREEN, A.R. (1981). Behavioural effects of central and peripheral injection of various analogues and metabolites of thyrotrophin releasing hormone (TRH). *Neuropharmacology*, **20**, 947–957.
- HEAL, D.J., STOODLEY, N., ELLIOTT, J.M., MARSDEN, C.A., BENNETT, G.W. & YODIM, M.B.H. (1987). Behavioural and biochemical evidence for the release of noradrenaline in mouse brain by TRH and some of its biologically stable analogues. *Neuropharmacology*, **26**, 313–322.
- JOHNSON, J.V., FONE, K.C.F., TOPHAM, I., BENNETT, G.W. & MARSDEN, C.A. (1989). Comparative motor behavioural effects of TRH-related peptides following intrathecal administration in rats. Recent advances in the biomedical significance of thyrotrophin releasing hormone (TRH). *New York Acad. Sci.*, (in press).
- KALIVAS, P.W. (1982). Histamine induced arousal in the conscious and pentobarbital pre-treated rat. *J. Pharmacol. Exp. Ther.*, **222**, 37–42.
- KALIVAS, P.W. & MILLER, J.S. (1984). Substance P modulation of dopamine in the nucleus accumbens. *Neurosci. Letts.*, **48**, 55–59.
- KALIVAS, P.W., NEMEROFF, C.B. & PRANGE, A.J. JR. (1984). Neurotensin microinjection into the nucleus accumbens antagonises dopamine induced increase in locomotion and rearing. *Neuroscience*, **11**, 919–930.
- KALIVAS, P.W., STANLEY, D. & PRANGE, A.J. JR. (1987). Interaction between thyrotrophin releasing hormone and the mesolimbic dopamine system. *Neuropharmacology*, **26**, 33–38.
- LIGHTON, C., MARSDEN, C.A. & BENNETT, G.W. (1984). The effects of 5,7 dihydroxytryptamine and p-chlorophenylalanine on thyrotrophin releasing hormone in regions of the brain and spinal cord of the rat. *Neuropharmacology*, **23**, 55–60.
- LIN, M.T., CHAN, H.K., CHEN, C.F. & TEH, G.W. (1983). Involvement of both opiate and catecholaminergic

- receptors in the behavioural excitation provoked by thyrotrophin releasing hormone: comparisons with amphetamine. *Neuropharmacology*, **22**, 463–469.
- LOWRY, O.H., ROSEBROUGH, N.J., LEWIS-FARR, A. & RANDALL, R.J. (1951). Protein measurements with the folin-phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
- MARSDEN, C.A. & KING, B. (1979). The use of doppler shift radar to monitor physiological and drug induced activity patterns in the rat. *Pharmacol. Biochem. Behav.*, **10**, 631–635.
- METCALF, G. (1983). The neuropharmacology of TRH analogues. In *Thyrotrophin Releasing Hormone*, ed. Griffiths, E.C. & Bennett, G.W. pp. 315–326. New York: Raven Press.
- MIYAMOTO, M. & NAGAWA, Y. (1977). Mesolimbic involvement in the locomotor stimulant action of thyrotrophin releasing hormone (TRH) in rats. *Eur. J. Pharmacol.*, **44**, 143–152.
- MIYAMOTO, M., NARUMI, S., NAGAI, Y., SAJI, Y. & NAGAWA, Y. (1984). A TRH analogue (DN1417): motor stimulation with rearing related to catecholaminergic mechanisms in rats. *Neuropharmacology*, **23**, 61–72.
- OGAWA, N., MIZUNO, S., NUKINA, I., TSUKAMOTO, S. & MORI, A. (1983). Chronic thyrotrophin releasing hormone (TRH) administration on TRH receptors and muscarinic cholinergic receptors in CNS. *Brain. Res.*, **263**, 348–350.
- PAXINO, G. & WATSON, C. (1982). *The Rat Brain in Stereotaxic Coordinates*. Australia: Academic Press.
- POLLARD, H. & SCHWARTZ, J.C. (1987). Histamine neuronal pathways and their functions. *Trends Neurosci.*, **10**, 86–89.
- PRELL, G.D. & GREEN, G.P. (1986). Histamine as a neuro-regulator. *Ann. Rev. Neurosci.*, **9**, 209–254.
- ROSTENE, W.H., MORGAT, J.L., DUSSAILLANT, M., RAINBOW, T.C., SARRIEAU, A., VIAL, M. & ROSSELIN, G. (1984). *In vitro* biochemical characterisation and autoradiographic distribution of [³H] thyrotrophin releasing hormone binding sites in rat brain sections. *Neuroendocrinology*, **39**, 81–86.
- SHARIF, N.A. & BURT, D.R. (1983). Rat brain TRH receptors: kinetics, pharmacology, distribution and ionic effects. *Regul. Peptides*, **7**, 399–411.
- SHARP, T., BENNETT, G.W., MARSDEN, C.A. & TULLOCK, I.F. (1984). A comparison of the locomotor effects induced by centrally injected TRH and TRH analogues. *Regul. Peptides*, **9**, 305–315.
- SHARP, T., ZETTERSTROM, T., LJUNGBERG, T. & UNGERSTEDT, U. (1986). Effect of sulpiride on amphetamine induced behaviour in relation to changes in striatal dopamine release *in vivo*. *Eur. J. Pharmacol.*, **129**, 411–415.
- SIMASKO, S.M. & HORITA, A. (1985). Treatment of rats with the TRH analogue MK771: down regulation of TRH receptors and behavioural tolerance. *Neuropharmacology*, **24**, 157–165.
- TAYLOR, R.L. & BURT, D.R. (1982). Species differences in the brain regional distribution of receptor binding for thyrotrophin releasing hormone. *J. Neurochem.*, **38**, 1647–1656.

(Received October 21, 1988

Revised February 22, 1989

Accepted March 13, 1989)