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Modulation of 5-hydroxytryptamine-induced head-twitch response by drugs acting at GABA and related receptors

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1 The effects of drugs acting at the γ-aminobutyric acid (GABA) receptors and other chloride ionophore-related sites have been studied for their ability to modulate the head-twitch induced by 1-5-hydroxytryptophan (5-HTP) in the mouse.

2 The $GABA_a$ receptor agonists, muscimol, imidazoleacetic acid and 3-aminopropanesulphonic acid, produced a dose-related potentiation, while bicuculline inhibited the head-twitch. The $GABA_b$ receptor agonist, baclofen, produced dose-related inhibition.

3 Diazepam potentiated the head-twitch while the 'inverse' benzodiazepine receptor agonist ethyl-β-carboline-3-carboxylate inhibited the head-twitch. The antagonist Ro15-1788 also produced inhibition. Ro05-4864, a ligand for the benzodiazepine 'acceptor' site, potentiated the head-twitch.

4 Pentobarbitone and pentylenetetrazol potentiated the 5-HTP-induced head-twitch at low doses, changing to inhibition as the dose was increased. Picrotoxin in subconvulsant doses, produced only potentiation. More than one site may be involved in the action of these substances.

5 GABA, amino-oxyacetic acid and 1-2-4-diaminobutyric acid inhibited the head-twitch, while the GABA-depletor, 3-mercaptopropionic acid potentiated it.

6 Of all the agents tested, only muscimol produced head-twitching when given alone.

7 It was concluded that both $GABA_a$ and $GABA_b$ receptors modulate the head-twitch response to 5-HTP.

Introduction

5-Hydroxytryptamine (5-HT) receptor agonists induce a characteristic head-twitch response, the frequency of which is dose-dependent (Corne *et al.*, 1963). This effect provides a model for the study of central 5-HT receptor activation and appears to be due to activation of $5\text{-}HT_2$ receptors (Peroutka *et al.*, 1981; Ortmann *et al.*, 1982; Green *et al.*, 1983). Because it is simple to quantitate, it has proved an attractive model for the study of transmitter interactions with 5-HTergic mechanisms. Such studies have demonstrated a role for dopaminergic (Maj *et al.*, 1978) and noradrenergic (Ortmann *et al.*, 1981; Handley & Brown, 1982; Handley & Singh, 1984a) mechanisms in the modulation of the 5-HTergic head-twitch response.

The potential involvement of γ-aminobutyric acid (GABA) has so far been little studied. GABA itself induces spontaneous head-twitching following peripheral administration to the rat and rabbit, but is ineffective in the mouse (Smialowski *et al.*, 1980). The $GABA_a$ receptor agonist, muscimol, has also been reported to induce the head-twitch (Scottie de Carolis & Massotti, 1978), as have certain benzodiazepines (Nakamura & Fukushima, 1976). The GABA and benzodiazepine effects at least were prevented by 5-HT antagonists (Nakamura & Fukushima, 1976; Smialowski *et al.*, 1980). Although the effects of benzodiazepines were formerly attributed to a direct action at 5-HT receptors (Nakamura & Fukushima, 1976), the close association between subpopulations of benzodiazepine and GABA receptors has since been elucidated (Study & Barker 1981; Skerritt *et al.*, 1982). The head-twitch inducing effects of GABA, muscimol and the benzodiazepines therefore point to a potential role for GABA receptors in the control of this response.

We have therefore investigated the effects of a range of drugs acting at GABA receptors and at other receptors on the GABA-chloride ionophore complex. These agents were tested for their ability to induce head-twitching when given alone and for their ability

to modulate 1-5-hydroxytryptophan (5-HTP) induced head-twitching behaviour. Some of these results have appeared in preliminary form (Handley & Singh, 1984b; 1985).

Methods

Animals

Male albino mice (MF1 strain, bred in our laboratories) weighing between 20 and 30 g were kept in the experimental room in groups of 25 (from the same birth cohort) on an 08 h 00 min to 20 h 00 min light-dark cycle at constant temperature (21 ± 1°C) for at least 5 days before the experiment. All behavioural studies were performed between 09 h 00 min and 18 h 00 min.

Induction of a head-twitch behaviour

One hour before experiment, mice from the same stock cage were placed in small sawdust lined polythene cages in groups of three. The third mouse was included only because head-twitching is reduced when there are only 1–2 mice per cage (Boulton & Handley, 1973). This mouse performed no further part in the experiment. The remaining pair received carbidopa (9 mg kg^{-1}, s.c.) followed 15 min later by 5-HTP (200 mg kg^{-1}, i.p.).

Analysis of drug effects

Drugs were injected i.p. 15 min before 5-HTP (exceptions given in Table 1). One mouse from each pair received the test drug and the other the injection vehicle. Twitches from the two mice were counted in parallel for 5 min starting 20 min post 5-HTP. This procedure compensates for between run variability (Handley & Brown, 1982). There were at least 6 pairs per experimental group. Drug effects relative to controls were assessed by paired t test carried out on the raw data. Potency was expressed as ID_{50} (dose producing a 50% inhibition relative to controls) or ED_{200} (dose producing a response frequency twice that of the controls) from log dose-respose regression analysis, where response = test mouse head-twitch frequency expressed as a % of that in paired control mouse (Handley & Brown, 1982). A minimum of four dose levels was investigated for each drug.

The highest dose of each drug which potentiated the 5-HTP-induced head-twitch was administered alone to determine whether it induced twitching on its own. Mice were observed for 30 min immediately after injection.

Solutions and drugs

Drugs were dissolved in saline (0.9% w/v NaCl) except the following: bicuculline (dissolved in 0.01N HCl); diazepam (commercial product Valium diluted in saline immediately before use); Ro15-1788 and Ro05-4864 (suspended in saline with 2 drops of Tween 80); ethyl-β-carboline-3-carboxylate (dissolved in saline by adding minimum amount of 1N HCl); pentobarbitone (commercial product, Sagatal, diluted in saline immediately before use); phenobarbitone (dissolved in saline by adding minimum amount of 1N NaOH).

The drugs were obtained from the following sources: GABA, amino-oxyacetic acid (AOAA), bicuculline methobromide, 1-2,4-diaminobutyric acid (DABA), 3-mercaptopropionic acid (3-MPA), muscimol, imidazoleacetic acid (IMAA), 3-aminopropanesulphonic acid (3-APS), pentylenetetrazol, picrotoxin and 1-5-hydroxytryptophan (5-HTP) were all obtained from Sigma, Poole, Dorset; Ro15-1788 ethyl 8-fluoro-5, 6-dihydro-5-methyl-6-oxo-4 H-imidazo [1,5-a] [1,4] benzodiazepine-3-carboxylate, Ro05-4864 7-chloro-1,3-dihydro-1-methyl-5-(4′-chlorophenyl)-2H-1,4-benzodiazepine-2-one and diazepam (Valium; Roche products, Welwyn Garden City, Herts); baclofen (Geigy pharmaceuticals, Macclesfield, Cheshire); car-

Table 1 Drug administration

Drug	*Route of administration*	*Time injected before 5-HTP*
GABA	i.c.v*	0
AOAA	s.c.	6 h
DABA	s.c.	24 h
3-MPA	i.p.	0
Muscimol	i.p.	0
IMAA	i.p.	30 min
Pentylenetetrazol	s.c.	0

* by the method of Brittain & Handley (1967).
GABA, γ-aminobutyric acid; AOAA, amino-oxyacetic acid; DABA 1-2,4-diaminobutyric acid; 3-MPA; 3-mercaptopropionic acid; IMAA; imidazoleacetic acid.

Table 2 Drugs potentiating the head-twitch response to 5-hydroxytryptophan (5-HTP) linear regression (log dose)

Drug	*Dose-range tested* (*mg kg*$^{-1}$)	P	r	ED_{200} (*mg kg*$^{-1}$)	*95% confidence limits* (*mg kg*$^{-1}$)
3-MPA	2.50– 10.00	<0.005	0.910	6.20	2.10– 18.30
Muscimol	0.12– 0.50	<0.001	0.983	0.26	0.19– 0.36
IMAA	12.50– 50.00	<0.02	0.921	16.02	9.33– 25.85
3-APs	25.00–200.00	<0.037	0.945	83.45	45.54–152.90
Diazepam	0.25– 1.00	<0.016	0.936	0.70	0.28– 1.80
Ro05-4864	5.00– 10.00	<0.044	0.901	5.94	1.72– 10.49

For abbreviations, see Table 1; 3-APS, 3-aminopropanesulphonic acid.
P = Significance of least squares fit.
r = Correlation coefficient of least squares fit.
ED_{200} = dose to increase twitches to 200% of control levels.

bidopa (Merck, Sharp and Dohme, Hoddesdon, Herts); ethyl-β-carboline-3-carboxylate (βCCE, Glaxo, Greenford, Middx); pentobarbitone (Sagatal) and phenobarbitone (May and Baker, Dagenham, Essex). Drug doses in text refer to the weight of free base.

Results

Drugs potentiating the head-twitch response to 5-hydroxytryptophan

Table 2 shows ED_{200} values for drugs which potentiated the 5-HTP head-twitch. These comprised the GABA synthesis blocker 3-MPA, the $GABA_a$ receptor agonists muscimol, 3-APS and IMAA, and the benzodiazepine receptor ligands diazepam and Ro05-4864. The dose-range of 3-MPA tested was limited by the occurrence of convulsions at 15 mg kg^{-1} and the doses of IMAA and diazepam which could be tested were limited by sedation; however there was no loss of righting reflex at the highest doses of these latter drugs.

ED_{200} values could not be obtained for picrotoxin because convulsions occurred at 1.0 mg kg^{-1}; there was significant potentiation at 0.5 mg kg^{-1} (122.2 ± 8.0% of paired control; $P<0.05$) and lower doses had no significant effect.

Drugs inhibiting the head-twitch response to 5-hydroxytryptophan

Table 3 shows ID_{50} values for drugs which inhibited the head-twitch response. These comprised the GABA metabolism inhibitor AOAA and the GABA uptake blocker DABA as well as GABA itself, the $GABA_b$ receptor agonist baclofen, the benzodiazepine receptor ligands βCCE and Ro15-1788 and the barbiturate, phenobarbitone. Mice given 60.0 mg kg^{-1} phenobarbitone were heavily sedated but loss of righting reflex

Table 3 Drugs inhibiting the head-twitch response to 5-hydroxytryptophan: linear regression (log dose)

Drug	*Dose-range tested* (*mg kg*$^{-1}$)	P	r	ID_{50} (*mg kg*$^{-1}$)	*95% confidence limits* (mg kg^{-1})
GABA	12.50–100.00*	<0.002	0.906	35.50*	21.90–57.31*
AOAA	6.00– 24.00	<0.001	0.956	10.90	8.45–24.23
DABA	5.00– 20.00	<0.05	0.920	22.37	10.48–37.13
Baclofen	1.25– 5.00	<0.002	0.981	2.66	1.84– 3.86
Ro15-1788	2.50– 10.00	<0.01	0.908	13.06	6.56–26.00
βCCE	20.00– 80.00	<0.04	0.903	41.05	10.12–60.03
Phenobarbitone	15.00– 60.00	<0.001	0.974	18.05	12.16–26.76

For abbreviations, see Table 1; βCCE, ethyl-β-carboline-3-carboxylate.
P = Significance of least squares fit.
r = Correlation coefficient of least squares fit.
ID_{50} = dose to decrease twitches to 50% of control levels.
*μg administered i.c.v. by the method of Brittain & Handley (1967).

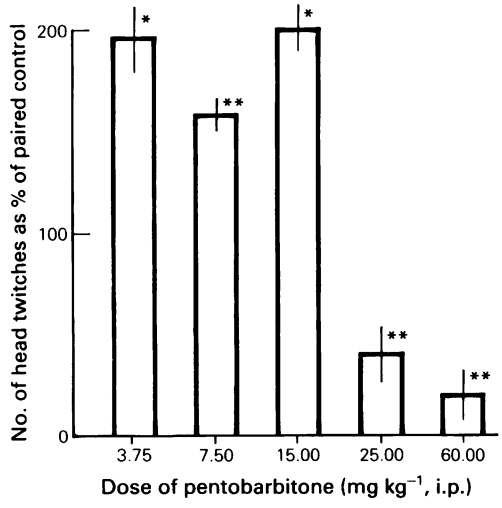

Figure 1 Effect of pentobarbitone on the 5-hydroxytryptophan (5-HTP)-induced head-twitch. All mice received 5-HTP (200 mg kg^{-1}, i.p.) and carbidopa (9 mg kg^{-1}, s.c.). Pairs of mice were assigned at random to control or test conditions and observed in parallel. Head-twitch frequency for test mouse was recorded as % of its paired control. Control mice received vehicle and the test mice received pentobarbitone. Results are the means of at least 6 determinations and vertical lines represent s.e.mean: * $P<0.05$; ** $P<0.001$.

did not occur at this dose and a lower dose (7.5 mg kg^{-1} i.p.) of this barbiturate had no significant effect on the head-twitch response.

No dose-response curve could be obtained for bicuculline because of convulsions occurring at 1.0 mg kg^{-1}; 0.5 mg kg^{-1} (30.1 ± 9.0% of paired control; $P<0.05$) significantly reduced the head-twitch but lower doses were ineffective.

Drugs with biphasic effects on the head-twitch response to 5-hydroxytryptophan

Low doses of pentobarbitone (3.75 to 15 mg kg^{-1}) potentiated the head-twitch as well as causing obvious excitement, whereas doses of 25 to 60 mg kg^{-1} reduced it (Figure 1). These higher doses were associated with sedation but the righting reflex was still present.

Pentylenetetrazol also produced biphasic effects with potentiation appearing at 20 mg kg^{-1} and inhibition at 40 mg kg^{-1} (Figure 2). Convulsions occurred at 50 mg kg^{-1}.

Drug effects alone

Only muscimol induced head-twitching when administered alone; 1.0 mg kg^{-1} produced 0.66 twitches per min over an observation period of 30 min immediately after injection.

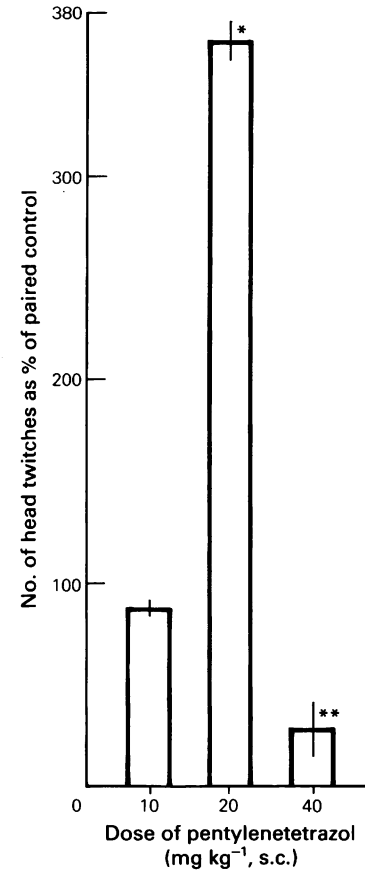

Figure 2 Effect of pentylenetetrazol on the 5-hydroxytryptophan (5-HTP)-induced head-twitch. All mice received 5-HTP (200 mg kg^{-1}, i.p.) and carbidopa (9 mg kg^{-1}, s.c.), pairs of mice were assigned at random to control or test conditions and observed in parallel. Head-twitch frequency for test mouse was recorded as % of its paired control. Control mice received vehicle and the test mice received pentylenetetrazol. Results are the means of at least 6 determinations and vertical lines represent s.e.mean: * $P<0.05$; ** $P<0.001$.

Discussion

There is evidence for two types of GABA receptor; $GABA_a$ linked to the chloride ionophore (see McBurney, 1984) and $GABA_b$ which is chloride ionophore-independent and is associated with inhibition of monoamine release (Bowery *et al.*, 1980; Hill & Bowery, 1981). Selective agonists for these two receptors had opposite effects on head-twitch frequency. Thus the $GABA_b$ agonist baclofen (Hill & Bowery, 1981; Bowery *et al.*, 1983) inhibited the head-twitch, while the $GABA_a$ receptor agonists muscimol, 3-APS

and IMAA (Hill & Bowery, 1981) produced potentiation. Both effects were dose-related. The inhibitory effect of the $GABA_a$ receptor antagonist bicuculline (Hill & Bowery, 1981) was consistent with the potentiating effect of corresponding agonists. The inhibitory effect of baclofen on 5-HTP-induced head-twitching has also been reported by Metz *et al.*, (1985), who found it to be ineffective against the direct agonist 5-methoxy *N,N*-dimethyltryptamine. This suggests that baclofen inhibits the head-twitch by reducing 5-HT release.

AOAA inhibits GABA-transferase (Wallach, 1961) and DABA inhibits neuronal GABA uptake (Sutton & Simmonds, 1974). These agents therefore raise endogenous GABA. Like GABA itself, they resulted in dose-related inhibition of the head-twitch. The results from the selective agonists suggest that this inhibition may be exerted through a predominant effect at the $GABA_b$ receptor. Spontaneous head-twitching after peripheral GABA administration in rat and rabbit (Smialowski *et al.*, 1980) may be due to a different balance of receptor activation in these species, favouring potentiating effects at the $GABA_a$ receptor. Interestingly, the GABA depleting agent (glutamic acid decarboxylase inhibitor) 3-MPA (Horton & Meldrum, 1973) induced potentiation, suggesting a tonic inhibitory role for GABA in the mouse.

Since $GABA_a$ receptors are part of a multi-receptor complex containing benzodiazepine and barbiturate receptors (see Olsen, 1981), ligands at these latter sites should have predictable effects on the 5-HTP induced head-twitch. Benzodiazepine receptor ligands showed the expected profile. Diazepam potentiated head-twitching (as reported by Nakamura & Fukushima, 1977) and the 'inverse agonist' βCCE (Polc *et al.*, 1982) inhibited it. Ro15-1788 is a benzodiazepine with antagonistic effects against both classic benzodiazepines and 'inverse agonists' (Hunkeler *et al.*, 1981). Ro15-1788 inhibited the head-twitch with four times the potency of βCCE. This could be interpreted either as being due to blockade of the effects of ongoing release of the putative endogenous ligand, or as due to intrinsic partial inverse agonist properties (File *et al.*, 1982).

A second benzodiazepine binding site has recently been described which appears to be non-neuronal, and has been suggested to be a silent 'acceptor' rather than a receptor (Richards *et al.*, 1982). The preconvulsant Ro05-4864 shows selectivity for this site *in vitro*. Ro05-4864 potentiated the head-twitch with a potency approximately one tenth that of diazepam; however it has recently been shown to bind to the picrotoxin/barbiturate site (Simmonds, 1984). The potentiating effect of Ro05-4864 may therefore not be due to an effect at the so called benzodiazepine 'acceptor' site.

The third component of the chloride ionophore receptor complex binds picrotoxin and barbiturates (Ticku & Olsen, 1978). Barbiturates prolong the duration of chloride channel opening (Huang & Barker, 1980) and enhance GABA-mediated responses (Nicoll *et al.*, 1975). By analogy with the effect of $GABA_a$ receptor agonists and benzodiazepine receptor ligands, the predicted effect of barbiturates would be to increase head-twitching frequency. However, the early work of Corne *et al.*, (1963) showed a wide range of barbiturates to be inactive up to very high doses. In the present experiments both pentobarbitone and phenobarbitone had marked effects. The difference from the results of Corne *et al.* (1963) probably lies in their use of a quantal (number of mice/group showing head-twitch) rather than a graded (head-twitch rate) response as a measure of head-twitch intensity, combined with our use of a pretreatment (200 mg kg^{-1} 5-HTP + 9 mg kg^{-1} carbidopa) which resulted in head-twitching in all mice. Phenobarbitone binds only weakly to the GABA-linked barbiturate receptor and, unlike pentobarbitone, does not potentiate GABA or benzodiazepine binding; pentobarbitone on the other hand is a potent ligand for this site (Thyagarajan *et al.*, 1983). The potentiation shown by pentobarbitone would be the predicted effect of agonist activity at the GABA-linked barbiturate receptor. The inhibitory effect shown by both barbiturates indicates the possibility of a second site of barbiturate action, perhaps that associated with inhibition of transmitter release (Fung & Fillenz, 1984).

The effect of two further ligands for the picrotoxin/barbiturate site, picrotoxin and pentylenetetrazol (see Ticku & Maksay, 1983) were similarly complex. The testable dose-range of picrotoxin was narrow because of its steep dose-response curve for convulsant activity. The highest subconvulsant dose caused potentiation while lower doses were inactive. This was unexpected since picrotoxin is also a chloride channel blocker (Simmonds, 1980) and would thus be predicted to cause inhibition, as did the GABA antagonist bicuculline. Pentylenetetrazol is also a convulsant but less potent than picrotoxin. For this compound high sub-convulsant doses did produce inhibition but potentiation was seen at lower doses. This may again indicate multiple sites of action.

Of all the drugs tested only muscimol caused detectable head-twitching when given alone. Muscimol is the most selective agonist for $GABA_a$ receptors of those used here (Hill & Rowery, 1981), so would be the least likely to antagonize its own effects through an opposing action at $GABA_6$ receptors.

In conclusion, GABAergic mechanisms appear to play an important role in the modulation of this 5-HT-induced behaviour. This could have clinical significance. Head-twitching is induced by hallucinogens (Corne & Pickering, 1987) and hallucinatory episodes have been reported after baclofen withdrawal (e.g. Stein, 1977), pentylenetetrazol (see Lal & Emett-

Oglesby, 1983), and muscimol (Theobald *et al.*, 1968) administration.

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