On the central noradrenergic mechanism involved in haloperidol-induced catalepsy in the rat

U. M. H. AL-SHABIBI, N. S. DOGGETT*, *Department of Pharmacology, Welsh School of Pharmacy, UWIST, Cardiff, U.K.*

It has been suggested that the ability of a neuroleptic agent to induce catalepsy in laboratory animals is related to drug-induced extrapyramidal side effects in man (see reviews by Fog, 1972; Hornykiewicz, 1973; Marsden, 1975). The potent cataleptogenic neuroleptics, especially those of the phenothiazine and butyrophenone series have been shown to have both central antidopaminergic and antinoradrenergic actions (Andén, Butcher & others, 1970; Sedvall, Fyrö & others, 1975). However the more selective antidopaminergic neuroleptics, such as pimozide, are observed to exert only limited extrapyramidal side-effects in man (Baro, van Lommel & others, 1972; Pinder, Brogden & others, 1976) and catalepsy in animals (Costall & Naylor, 1975). The purpose of this study is to investigate noradrenergic involvement in haloperidol-induced catalepsy using various agents which are known to interfere primarily with brain noradrenergic mechanisms.

Male Wistar rats, 200–250 g, were used in groups of 5 or 6 and injected intraperitoneally or intravenously with various doses of FLA 63 (bis(4-methyl-1,1-homopiperazinylthiocarbonyl)disulphide) (Astra), clonidine HCl (C. H. Boehringer), yohimbine HCl (Sigma), phentolamine HCl (Ciba) or phenoxybenzamine HCl (SKF) before a submaximal cataleptogenic dose of haloperidol. Animals were tested for the presence of catalepsy 1 h after haloperidol (or the α-adrenoceptor agonist or antagonist) administration and scoring continued at hourly intervals for 5 h. Both forelegs were placed on a 10 cm high bar, 1·2 cm in diameter, and the reaction time (time taken for the rat either to withdraw the forelegs completely or to climb over the bar) was recorded. A cut-off time of 60 s was employed. Whole brain noradrenaline and dopamine were measured according to Shellenberger & Gordon (1971).

The dopamine β-hydroxylase inhibitor FLA-63 induced catalepsy in the rat at 25 mg kg^{-1}; while 15 mg kg^{-1} was without cataleptogenic effect (Table 1). As with haloperidol, the catalepsy induced by FLA-63 was associated with marked behavioural sedation. The drug, when used in high doses, also potentiated the catalepsy produced by a submaximal cataleptogenic dose of haloperidol (1 mg kg^{-1}) at 2 h ($P < 0.01$) and 4 h ($P < 0.05$) after neuroleptic administration. Animals treated with FLA-63 (25 mg kg^{-1}) alone showed a 53% reduction in whole brain noradrenaline

* Correspondence.

Table 1. *Mean (±s.e.m.) values in seconds for cataleptic after haloperidol† and various adrenoceptor agonists and antagonists scored by the bar-method, maximum bar-time being 60 s.*

Drug, dose (mg kg^{-1})	n	Average intensity of catalepsy (s ± s.e.m.) for 1st h	2nd h	3rd h	4th h	5th h
1-Sal + Hal	6	29·17 ± 6·67	25·8 ± 5·23	36·67 ± 6·15	25 ± 3·65	25·8 ± 4·9
FLA 63 (25)	6	13·3 ± 1·67	16·67 ± 1·67	20·8 ± 3·75	23·3 ± 5·87	23·3 ± 4·4
FLA 63 (15) + Hal	5	22 ± 4·06	33 ± 7·52	32 ± 8·15	30 ± 8·22	25 ± 8·94
FLA 63 (25) + Hal	5	40 ± 8·37	56 ± 4**	41 ± 7·8	40 ± 5·48*	33 ± 11·14
2-Sal + Hal	6	16·67 ± 2·79	17·5 ± 3·1	21·67 ± 4·22	16·67 ± 3·8	11·67 ± 2·79
Phenoxyben (20)	5	4 ± 1·7	6·4 ± 1·57	3·6 ± 0·6	2·6 ± 1·12	2 ± 0·9
Phenoxyben (10) + Hal	6	22·5 ± 4·43	25 ± 2·24	24·17 ± 2·71	18·33 ± 1·67	13·3 ± 2·47
Phenoxyben (20) + Hal	6	30 ± 2·58**	50 ± 6·32**	30 ± 3·65	26·67 ± 4·22	16·67 ± 3·07
Clonidine (0·5) + Hal	6	2·5 ± 1·17**	5 ± 2·58**	5·83 ± 2·39**	15 ± 2·58	15 ± 1·83
Clonidine (0·1) + Hal	6	3·33 ± 1·67	5·83 ± 1·54**	6·67 ± 1·67**	3·3 ± 1·05**	2·5 ± 1·71**
3-Sal + Hal	6	21·67 ± 3·33	28·3 ± 3·57	35 ± 6·06	21·67 ± 4·01	19·17 ± 3·75
Phentol (2) + Hal	5	15 ± 4·18	19 ± 3·32	26 ± 2·92	25 ± 4·7	23 ± 2·55
Phentol (5) + Hal	5	16 ± 4	18 ± 4·9	28 ± 9·57	30 ± 8·9	24 ± 11·1
Phentol (10) + Hal	5	18 ± 4·9	27 ± 8·89	32 ± 7·35	30 ± 7·75	26 ± 1·87
Phentol (20) + Hal	5	15 ± 2·74	24 ± 9·41	31 ± 9	22 ± 3·74	21 ± 1·87
Yohimb (1) + Hal	5	9 ± 1·87**	12 ± 1·22**	12 ± 2**	14 ± 1·87	24 ± 2·92
Yohimb (2) + Hal	5	7 ± 2**	10 ± 1·58**	12 ± 2·55**	23 ± 3·74	24 ± 5·1
Yohimb (3) + Hal	5	0	0	2 ± 2**	5 ± 1·58**	7 ± 2·55**
Yohimb (10) + Hal	5	0	0	0	0	0

† Haloperidol (Hal) was always administered at a submaximal cataleptic dose of 1 mg kg^{-1} (i.p.) 30 min after an intreperitoneal injection of phenoxybenzamine (phenoxyben, clonidine, phentolamine (phentol) or yohimbine (yohimb), or 1 h after an intravenous injection of FLA 63. Sal-Saline 0·9% w/v.

Significance (* $P < 0.05$) (** $P < 0.01$) according to the Mann–Whitney comparisons of each individual group with their own daily controls (Sal + Hal).

n = number of rats.

Table 2. *Effect of FLA 63 on depletion of brain noradrenaline as estimated in whole brain 5 h after drug administration.*

Treatment	n	Dose mg kg^{-1}, i.v.	Noradrenaline	Dopamine
Saline	4	—	329 ± 10·46	902·75 ± 131
FLA 63	6	25	176·67 ± 4·33***	975 ± 44·06

***$P < 0.001$ as compared with the saline treated animals by the Student's *t*-test.
n = number of rats.

concentrations ($P < 0.001$) 5 h after administration while dopamine concentrations were not significantly affected (Table 2).

Phenoxybenzamine showed no significant effect on haloperidol catalepsy at a dose of 10 mg kg^{-1} but potentiated it ($P < 0.01$) the first 2 h at the higher dose of 20 mg kg^{-1}. At 20 mg kg^{-1}, phenoxybenzamine alone produced catalepsy and sedation but both were much less marked than those observed after FLA-63. Phentolamine produced no such potentiation of haloperidol catalepsy when used at similar doses (2–20 mg kg^{-1}), in contrast, a slight reduction was seen during the first 2 h after administration of neuroleptic. Furthermore, no catalepsy and only slight sedation was seen after phentolamine alone (cf. phenoxybenzamine).

Yohimbine, a potent presynaptic noradrenaline receptor blocker which accelerates noradrenaline and dopamine turnover (Andén & Grabowska, 1976), produced a dose-dependent antagonism of haloperidol catalepsy over the dose range 1–10 mg^{-1}, complete antagonism being observed at the highest dose. The yohimbine-treated animals also showed periodic hyperactivity and stereotyped sniffing at the higher doses.

Animals receiving clonidine showed some behavioural sedation but they were easily aroused when handled. This effect was mostly observed following 0·1 mg kg^{-1} when antagonism of haloperidol catalepsy pesisted for 5 h ($P < 0.01$). The higher dose of 0·5 mg kg^{-1} was only effective in antagonizing catalepsy during the first 3 h and increasing the dose further to 1 mg kg^{-1} produced marked sedation associated with abnormal postures unrelated to those usually observed during cataleptic behaviour.

Our results show that specific depletion of central noradrenaline by FLA-63 induces cataleptic responses and potentiates haloperidol catalepsy. Honma & Fukushima (1977), however, did not report any cataleptogenic effects of FLA-63. Further evidence for the involvement of noradrenaline mechanisms in haloperidol catalepsy has been put forward by Pycock (1977) who showed that 6-hydroxydopamine lesions at birth and bilateral electrolytic lesions in the locus coeruleus potentiated haloperidol catalepsy in the rat.

According to Doxy, Smith & Walker (1977) the α-adrenoceptor blocking agents can be classified as preferentially presynaptic (e.g. yohimbine), preferentially postsynaptic (e.g. phenoxybenzamine), or pre- and post-synaptic (e.g. phentolamine). In the present experiments, phenoxybenzamine potentiated haloperidol catalepsy, yohimbine antagonized it, whereas phentolamine produced no significant effect. This suggests that a decreased availability of noradrenaline at the postsynaptic receptor, brought about by postsynaptic blockade, predisposes towards cataleptic behaviour, whereas an increased availability caused by presynaptic noradrenaline receptor blockade has the opposite effect. This would explain the lack of activity of phentolamine and would account for the fact that yohimbine-treated animals exhibited hyperactivity and stereotyped movements.

The α-adrenoceptor agonist clonidine antagonized haloperidol catalepsy at low doses but paradoxically some behavioural sedation was seen. These results also support the role of noradrenaline in the mediation of haloperidol catalepsy although they differ from those of Andén, Strombom & Svensson (1973) who found that clonidine was ineffective in altering the motor activity of reserpinized mice, although it potentiated apomorphine stimulation of the suppressed motor activity. It may be that different mechanisms are involved in the production of reserpine catalepsy, although differences in the animal models, doses, or animal species cannot be excluded for such discrepancies.

In conclusion it seems that noradrenergic mechanisms can probably play more than a modulatory role in haloperidol-induced catalepsy. Depletion of noradrenaline stores, as with FLA-63, induces catalepsy by itself and potentiates haloperidol-induced catalepsy, while stimulation of central noradrenergic mechanisms either directly (e.g. with low doses of clonidine), or by increasing noradrenaline turnover (e.g. with yohimbine), effectively antagonized it. The clinical significance of the present findings remains to be demonstrated.

We are grateful to Sigma, Astra, SKF and C. H. Boehringer for their generous supply of drugs.

February 1, 1978

REFERENCES

ANDÉN, N. E., BUTCHER, S. G., CORRODI, H., FUXE, K. & UNGERSTEDT, U. (1970). *Eur. J. Pharmac.*, **11**, 303–314.
ANDÉN, N.-E. & GRABOWSKA, M. (1976). *Ibid.*, **39**, 275–282.
ANDÉN, N.-E., STROMBOM, U. & SVENSSON, T. H. (1973). *Psychopharmac.*, **29**, 289–298.
BARO, F., VAN LOMMEL, R., DOM, R. & DE MESMAECHER, L. (1972). *Acta Psychiat. belg.*, **72**, 199–214.
COSTALL, B. & NAYLOR, R. J. (1975). *Psychopharmac.*, **43**, 69–74.

DOXY, J. C., SMITH, G. F. C. & WALKER, J. M. (1977). *Br. J. Pharmac.*, **60**, 91–96.

FOG, R. (1972). *Acta Neurol. Scand.*, **50**, *Suppl.* 1.

HONMA, T. & FUKUSHIMA, H. (1977). *Neuropharmac.*, **15**, 601–607.

HORNYKIEWICZ, O. (1973). *Br. Med. Bull.*, **29**, 172.

MARSDEN, C. D. (1975). In: *Modern Trends in Neurology*, Vol. 6, 141–166. Editor: D. Williams. London: Butterworths.

PINDER, R. M., BROGDEN, R. N., SAWYER, P. R., SPEIGHT, T. M., SPENCER, R. & AVERY, G. S. (1976). *Drugs*, **12** (1), 1–40.

PYCOCK, C. (1977). *Naunyn-Schmiedebergs Arch. Pharmac.*, **298**, 15–22.

SEDVALL, G., FYRÖ, B., NAYBACK, H. & WIESEL, F. A. (1975). In: *Advances in Neurology*, I, p. 133. Editors: Calne, D., Chase, T. & Barbeau, A. New York: Raven Press.

SHELLENBERGER, M. K. & GORDON, J. H. (1971). *Analyt. Biochem.*, **39**, 356–372.

LETTER TO THE EDITOR

Lipophilicity and bitter taste

R. J. GARDNER, *Group R&D Laboratory, Harp Lager Ltd., Manor Park, Alton, Hants GU34 2PS, U.K.*

In a recent communication, Schober, Bowers & Smith (1978) commented on the 'Low stereospecificity of quinine taste receptors'. In the light of their observations I would like to raise a number of points which may be of significance in the perception of a bitter taste and, perhaps, of flavours in general.

The range of compound types which induce the perception of bitterness in man is wide and includes alkaloids (Schober & others, 1978), amino acids and peptides (reviewed by Guigoz & Solms, 1976), polyphenolic compounds (Horowitz & Gentili, 1969; Esaki, Kamiya & Konishi, 1977), various compounds and their analogues, derived from *Humulus lupulus* L. (Whitear, 1969; Molyneux & Eggling, 1969; Gienapp & Schroder, 1975) and terpenes (Kubota & Kubo, 1969). The physical processes occurring when man perceives a given taste (or odour) are largely unknown, but, in general terms, some interaction between the tastant and a receptor-site seems likely. Although a 'bitter-sensitive' protein has been isolated from porcine tongues (Dastoli, Lopiekes & Doig, 1968), the view that it is a taste recognition molecule has now been abandoned (Price & Desimone, 1977). As an alternative to a protein it has been suggested (Kurihara, 1973) that membrane lipids are the bitter receptor sites.

Thus the nature of the receptor-site responsible for perception of bitterness is undecided and the structural features which make a given compound bitter unknown. However, Kubota & Kubo (1969) studying a series of diterpenes, found that a necessary prerequisite for these compounds to be bitter was the presence of a proton-donor group and a proton-acceptor group 'within a distance of about 1·5 Å making it possible to form an *intra*-molecular hydrogen bond'. They referred to this donor-receptor pair as the 'bitterness unit' and suggested that it interacted with the active site on the receptor, thus fixing the bonding units of the site at about 1·5 Å apart. However, the work of Schober & others (1978) is at variance with this conclusion. Thus although the quinines studied have appropriate acceptor and donor groups (quinuclidine N and C9 hydroxyl), they are further apart than the model proposes. Observations on the structures of bitter sugar analogues (Birch & Lee, 1976) also do not support the Kubota & Kubo (1969) model. One explanation for this difference lies in the possibility that there is more than one type of receptor. The fact that many people cannot taste phenylthiocarbamide, but can taste other bitter compounds, does imply the existence of at least two bitter receptor sites (Price & Desimone, 1977).

Even if there are a number of different receptor sites, the observation (Kubota & Kubo, 1969) that the presence of an *intra*-molecular hydrogen bond correlates with bitterness is puzzling from the structural point of view; i.e. such a bond has to be broken for interaction with the receptor site. The energies involved are not great, but it is known (Schallenberger, 1963) that hydrogen-bonding between hydroxyl groups in sugars restricts their sweetness. I would suggest that the correlation between an *intra*-molecular hydrogen bond and bitterness, in the terpenes, can be explained in terms of the effect of this feature on the physical properties of these compounds. Thus any *intra*-molecular hydrogen bonding in a molecule will effectively increase its lipophilicity compared with similar structures where *inter*-molecular hydrogen bonding occurs. Since bitter molecules probably have to penetrate cells (or at least cell walls) of the tongue to elicit a bitter response (Price & Desimone, 1977), *intra*-molecularly hydrogen bonded terpenes are more likely to reach the site of action than those bonded *inter*-molecularly.

The significance of lipophilicity to the perception of bitterness is illustrated by many observations:

1. The bitterness of a series of bitter compounds