Anti-aggressive action of dopamine-β-hydroxylase inhibitors in mice

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The development of aggressiveness in male mice upon isolation (Allee 1942; Scott 1946; Yen, Stanger & Millman, 1959) has been utilized for the screening of potential anxiolytic compounds (Janssen, Jagenau & Niemegeers, 1960; Valzelli, Giacalone & Garattini, 1967) as well as for studies attempting to correlate the behavioural changes produced by isolated housing with biochemical changes in the central nervous system (Essman, 1969; Garattini, Giacalone & Valzelli, 1969). The role of the biogenic monoamines in the aggressive state has been extensively studied and there are observations indicating a decreased turnover of 5-hydroxytryptamine (5-HT) (Garattini & others, 1969) and noradrenaline (Welch & Welch, 1969) in isolated mice compared to those housed in groups. However, the relation between these biochemical changes and the aggressiveness is still unclear since there are mice strains which do not show any changes in the turn-over of these amines although the mice become aggressive on isolation (Goldberg, Dubnick & others, 1973a; Goldberg, Insalaco & others, 1973b). Irrespective of this controversy, monoaminergic neurons may be involved in the acute state of aggressive behaviour. The role of noradrenaline in the aggressive response can be examined by a selective blockade of its enzymatic formation by inhibition of the dopamine-β-hydroxylase (DBH), the enzyme catalysing the hydroxylation of dopamine to noradrenaline in the noradrenergic neurons. The development in our laboratories of potent DBH inhibitors (Carlsson, Corrodi & others, 1970; Florvall & Corrodi, 1970) has made it possible to reduce the brain concentration of noradrenaline without lowering that of dopamine and 5-HT. In the present study we have examined the effect of two potent DBH inhibitors, 4-methyl-1-homopiperazinedithiocarboxylic acid (FLA 57) and bis(4-methyl-1-homopiperazinylthiocarbonyl)disulphide (FLA 63), on the aggressive behaviour of isolated mice and on the aggressiveness produced by L-dopa in normal mice.

Male albino mice (NMRI) were housed isolated for 6 weeks to get highly aggressive animals. The strength of the aggressive behaviour was measured according to the method of Valzelli & others (1967) with the exception that two mice were placed together instead of groups of three. Aggressive behaviour was scored before the injection of the DBH inhibitors and at various times thereafter. The animals were always used as their own control. At least 6 pairs were tested at each time. The noradrenaline concentrations in the mouse brain were determined according to Anton & Sayre (1962). The aggressive behaviour produced by L-dopa (100 mg kg^{-1}) in mice pre-treated with phenidprazine (1·25 mg kg^{-1}) (Everett, Davis & Toman, 1959) was scored by a scale from 1 to 3, in which 3 denotes the maximal response the end point of which is aggressive behaviour (Ross, Renyi & Ögren, 1972).

As shown in Fig. 1 the two DBH inhibitors strongly antagonized the aggressive behaviour in the isolated mice at doses reducing the noradrenaline concentration in the mouse brain (Table 1). FLA 63 was more potent than FLA 57 which is in accordance with the effects on the brain noradrenaline. The anti-aggressive action was obtained earlier than the measured fall in the brain noradrenaline, which indicates that the decrease in noradrenaline concentration was not directly related to the anti-aggressive effect. A better relation probably exists between the inhibition of DBH in the

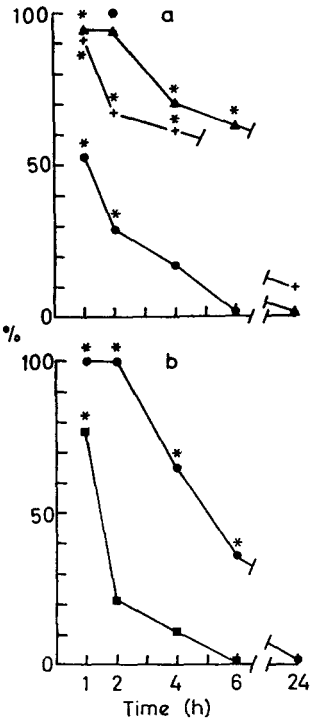

FIG. 1. Effect of a-FLA 57 and b-FLA 63 on the aggressiveness of isolated male mice. The aggressive responses of the paired mice was examined before the injection and at different times thereafter. Each value is the mean of responses from at least 6 × 2 mice and expressed as % block of the aggressiveness before injection. * denote significant block of the aggressive responses ($P<0.05$, Mann-Whitney U-test). ▲ 100 mg kg^{-1}, i.p., + 50 mg kg^{-1}, i.p., ● 25 mg kg^{-1}, i.p., ■ 10 mg kg^{-1}, i.p.

* Correspondence

Table 1. *Effect of FLA 63 and FLA 57 on the noradrenaline concentration in mouse brain.* Each value is the mean ± s.e.m. of at least 4 groups of 3 pooled mouse brains and expressed in % of the noradrenaline concentration in the control brains (0·384 ± 0·012 μg g^{-1}, n = 24). Grouped mice weighing 30–35 g were used.

Compound	Dose mg kg^{-1}, i.p.	Noradrenaline, % of control (±s.e.m.) 1	3	6	24 h
FLA 63	10	85±5	72±3*	88±1*	95±2
	25	70±3	42±8*	57±3*	94±3
FLA 57	25	82±9	67±3*	69±4*	—
	50	84±4	51±4*	52±6*	86±6

* $P<0·05$ (Student's *t*-test).

Table 2. *Effect of clonidine on the anti-aggressive action of FLA 63 in isolated male mice.* FLA 63, 20 mg kg^{-1}, s.c. was given 1 h before clonidine. The aggressiveness was tested 1 and 6 h after clonidine (saline) and was expressed in % of the responses before the first injection. Each value is the mean of the number (n) of paired mice noted.

Treatment	Dose mg kg^{-1}, i.p.	n	Aggressiveness, % 1	6 h
Saline	—	8	4	96
Clonidine	0·1	2	0	100
Clonidine	0·5	4	0	17*
Clonidine	10	2	0	0

* $P<0·05$ (Mann-Whitney U-test) compared with the control.

brain and the anti-aggressive action, which could mean that aggressive behaviour requires an intact synthesis of noradrenaline. However, it must be remembered that the same time courses will be found for any effect in which the presence of FLA 57 or FLA 63 in the brain is essential. To further strengthen the relation between the anti-aggressive effect of the DBH inhibitors and the inhibition of the synthesis of noradrenaline in the brain, (±)-*threo*-dihydroxyphenylserine (dops) (200 mg kg^{-1}) was injected intravenously 1 h after FLA 63, 20 mg kg^{-1}, and the aggressive responses were recorded 1 h later, whereupon the mice were immediately killed. According to the report of Creveling, Daly & others (1968) (±)-*threo*-dops is decarboxylated to noradrenaline in the mouse brain. However, under the conditions used in our experiment (±)-*threo*-dops failed to significantly increase the noradrenaline concentration (controls: 0·511 ± 0·052 μg g^{-1}, FLA 63: 0·326 ± 0·006 ($P<0·05$), FLA 63 + dops: 0·380 ± 0·029; each value is the mean ± s.e.m. of 5 × 3 mice) and did not reverse the effect of FLA 63 (FLA 63:22% and FLA 63 + dops: 13% of the control aggressiveness). The very low rate of the enzymatic decarboxylation of dops *in vivo* is probably the reason for this negative finding (cf. Goodwin, Johnson & others, 1972).

FLA 63 (10 mg kg^{-1}, s.c.) abolished the aggressive-like behaviour induced by L-dopa (100 mg kg^{-1}, i.p.) at the same dose which blocked aggression in isolated mice (mean scores of 4 groups of 3 mice: L-dopa: +3; FLA 63 + L-dopa: + 1).

Since the experiments with dops were negative, we examined clonidine, a central noradrenergic receptor agonist (Andén, Corrodi & others, 1970; Schmitt, Schmitt & Fenard, 1971), to see if it was able to reverse the blocking effect of FLA 63 on the aggressive behaviour. However, we found that clonidine itself was a potent antagonist of the aggressiveness in isolated mice with an ED50 value of 0·15 mg kg^{-1}, intraperitoneally (95% confidence limits: 0·12–0·20 mg kg^{-1} 1 h after the injection. It did not reverse the effect of FLA 63 but on the contrary increased the antagonism (Table 2).

Aggressive behaviour is certainly a complex phenomenon and results obtained by chemical manipulations must be interpreted cautiously. Although the anti-aggressive action of the DBH inhibitors was dose-related to the fall in the noradrenaline concentration in the mouse brain these metal chelating compounds may have other yet unknown effects resulting in this antagonism. Further studies with other DBH inhibitors of different structures may give more information on the potential relation between noradrenaline and aggressive behaviour in isolated mice. Hodge & Butcher (1975) recently reported that disulfiram, which is closely related to FLA 63, antagonized this behaviour.

The observation that clonidine antagonized the aggressive responses in isolated mice, but did not reverse the effect of FLA 63, seemingly contradicts the hypothesis that noradrenergic neurons are involved in this behaviour. However, Starke, Montel & others (1974) recently reported that clonidine is a much stronger agonist on the pre-junctional noradrenergic receptors than on the post-junctional receptors in the rabbit pulmonary artery. If this observation is also valid for central noradrenergic receptors the effect of clonidine in aggressive mice may be explained by stimulation of pre-synaptic receptors resulting in a decreased post-synaptic noradrenergic receptor activity. At high doses (>10 mg kg^{-1}) clonidine is reported to produce aggressive-like biting behaviour in grouped mice (Morpurgo, 1968) which could be due to direct stimulation of central post-synaptic noradrenergic receptors. However, it then remains to be explained why large doses of clonidine failed to reverse the anti-aggressive effect of FLA 63. Obviously more experiments have to be performed in order to answer the question as to whether noradrenergic neurons are involved in the aggressive behaviour in isolated mice.

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Apomorphine as an antagonist of the dopamine response from the nucleus accumbens

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The nucleus accumbens has been subjected to extensive investigation as a site at which dopamine and dopamine agonists are able to initiate hyperactivity (Pijnenburg & van Rossum, 1973; Elkhawad & Woodruff, 1975; Kelly, Seviour & Iversen, 1975; Pijnenburg, Honig & van Rossum, 1975; Costall & Naylor, 1975, 1976; Costall, Naylor & Pinder, 1976; Pijnenburg, Honig & others, 1976) and a number of models, based on the effect of dopamine in this area, have been proposed for the detection of both dopamine agonist (Iversen, Kelly & others, 1975; Kelly, 1975; Kelly, Miller & Neumeyer, 1975) and antagonist activity (Costall & Naylor, 1976). We have been particularly interested in a model proposed by Iversen and her colleagues in which 6-hydroxydopamine is injected into the nucleus accumbens to increase the sensitivity of the dopamine receptors in this area and render an animal more sensitive to the hyperactivity inducing effect of dopamine agonists. However, we find one major difficulty in interpretation of data from this model: following 6-hydroxydopamine injections into the nucleus accumbens apomorphine is shown to induce a marked locomotor response (Iversen & others, 1975) yet we find that apomorphine is not a stimulant of locomotor activity in normal rats and does not induce a hyperactivity when injected directly into the nucleus accumbens of normal animals (Costall, Naylor & Neumeyer, 1975a). Further, in our hands, injections of 6-hydroxydopamine into the nucleus accumbens (which fail to significantly modify dopamine content of the tuberculum olfactorium) fail to render animals sensitive to a hyperactivity component of the apomorphine effect, either when apomorphine is injected by a peripheral route or directly into the 6-hydroxydopamine-treated nucleus accumbens. However, if 6-hydroxydopamine is placed in the tuberculum olfactorium, apomorphine may then produce a hyperactivity (Costall & others, in preparation). This would tend to emphasise a point which has been raised by Iversen and her colleagues that the response to apomorphine they observed may

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