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FUROXANOBENZOFUROXAN, A SELECTIVE MONOAMINE OXIDASE INHIBITOR

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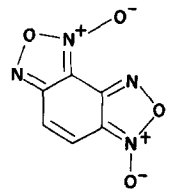
Abstract—Furoxanobenzofuroxan (FBF) has been previously described as an inhibitor of monoamine oxidase. Inhibition was reversible, and of a mixed competitive and non-competitive type, with a molar inhibition constant of 4×10^{-7} M. *In vivo*, the drug was selective in elevating the levels of indolealkylamines but not phenylethylamines. Low doses of FBF produced significant elevation of 5-hydroxytryptamine (5-HT) in the brain of the guinea-pig, but not in the heart and liver. Noradrenaline (NA) levels in these organs were not affected at the low dose. On the other hand, an equimolar dose of tranylcypromine caused elevation of NA in the heart and brain, but had no effect on 5-HT levels. FBF was shown to be a powerful blocking agent of 5-HT in the periphery, giving it a range of properties similar to those reported for the harmala alkaloids. However, no evidence was found that FBF shared the hallucinogenic properties of harmine; in fact, FBF appeared to block the central action of 5-hydroxytryptophan, a known hallucinogen. Thus, the 5-HT antagonist activity of FBF appeared to predominate over its action in elevating 5-HT levels due to inhibition of monoamine oxidase.

THE *in vitro* inhibition of monoamine oxidase (Monoamine: O_2 oxidoreductase (deaminating); E.C. 1.4.3.4.; MAO) by a series of oxazoles, oxadiazoles, benzoxadiazoles and related compounds has been studied recently.[1] An increase in inhibitory activity of the series was observed from the monocyclic oxadiazole, through the bicyclic benzoxadiazole to tricyclic compounds such as naphthofurazan and furoxanobenzofuroxan (I).



(I)

Furoxanobenzofuroxan (FBF) was the most potent inhibitor of the series, with an inhibition constant of 4×10^{-7} M. Further studies have now been carried out with FBF. In addition to studies on the *in vitro* and *in vivo* inhibition of MAO by FBF,

selected pharmacological properties possessed by the compound were compared with certain properties possessed by tranylcypromine, typical of the MAO inhibitors used clinically in angina pectoris, hypertension and depression.[2] FBF was also compared with harmine, a known MAO inhibitor and hallucinogen.[3]

EXPERIMENTAL

In vitro *MAO assay*. Measurement of MAO activity was carried out by the method of Wurtman and Axelrod[4] as has been described previously.[1] The substrate was ^{14}C-tryptamine (New England Nuclear; 10·7 mCi/mM) and the enzyme source was a washed mitochondrial fraction from rat liver.[5] The properties and use of this MAO preparation in inhibition studies have been described.[1]

MAO inhibition in vitro. Female guinea-pigs were injected intraperitoneally and after 4 hr, animals were decapitated, the heart, brain and liver excised, rinsed in distilled water to remove surface blood, weighed and frozen. Tranylcypromine and FBF were injected at 0 and 10 mg/kg and 0, 15 and 50 mg/kg, respectively. All animals were killed at approximately the same time of day to minimise errors due to circadian rhythms in brain amine levels.[6] 5-Hydroxytryptamine (5-HT) and noradrenaline (NA) levels in frozen tissue were measured fluorimetrically with an Aminco-Bowman spectrophotofluorometer.[7] Readings were linear in the range 0·01–2·5 μg 5-HT and 0·025–2·5 μg NA. Results were calculated as μg of amine present per g (wet weight) of tissue.

Antagonism of 5-HT. The isolated rat fundus strip[8] was set up in a 30 ml organ bath in de Jalon's solution at 37° and aerated with carbogen.[9] Muscle contractions were measured using an isotonic transducer and a pen recorder. Tension was applied to the muscle by means of a 1 g weight attached over a pulley. The cumulative method was used to obtain dose–response curves. Successive doses were added to the bath every 2 min, when responses were maximal.

Activity in the mouse twitch test. Corne and Pickering[10] tested a range of drugs with hallucinogenic activity in man and reported that they produce a characteristic head twitch response in mice. Animals were observed, in pairs, for 1 hr after injection with drugs intraperitoneally, and the number of twitch responses was recorded for each mouse during a 2 min period every 5 min. Each treatment group contained 10 mice.

RESULTS

FBF inhibited MAO in a mixed competitive and non-competitive manner, with a molar inhibition constant of 4×10^{-7}M[1]. Figure 1 shows the effects on MAO activity of increasing FBF concentration, using fixed amounts of enzyme and substrate. A simple hyperbolic plot was obtained.

Preincubation of enzyme with inhibitor caused only a slight increase in the amount of inhibition observed (Table 1). Apparently FBF was active without conversion to an "active principle" as has been suggested for iproniazid.[11] In addition, these results showed that FBF was not inactivated by the mitochondrial preparation.

When the initial reaction velocity was plotted against enzyme concentration in the presence of various concentrations of FBF, straight lines were obtained, converging on a single point (Fig. 2), a pattern indicating that FBF was a reversible inhibitor of MAO.[12] FBF could also be readily removed from its association with MAO by

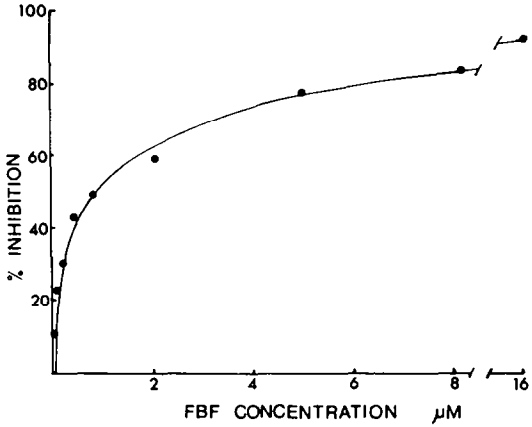


FIG. 1. Effect of increasing FBF on MAO activity. Duplicate assays contained 2×10^{-5} M tryptamine, 4 μg enzyme protein and FBF in the concentration range 4×10^{-8}–2×10^{-5} M. Results show percentage inhibition of enzyme activity.

TABLE 1. EFFECTS OF PREINCUBATION WITH FBF ON MAO ACTIVITY

Preincubation time (min)	% Inhibition I	II
0	43	41
1	49	40
2·5	43	40
5		43
10	49	
15	51	48

Assays performed in duplicate, contained 2×10^{-5} M tryptamine, 8×10^{-7} M FBF and 4 μg enzyme protein. Reaction was initiated after preincubation by the addition of substrate. Results are expressed as percentage inhibition of activity of the same enzyme sample assayed in the absence of inhibitor.

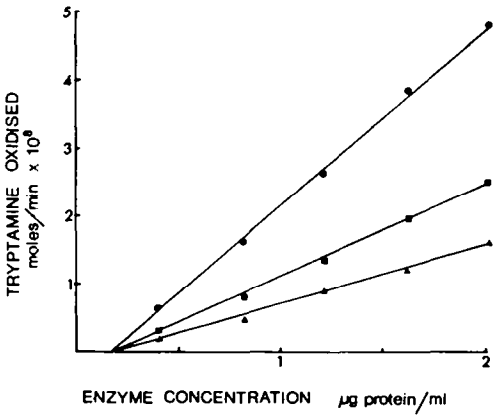


FIG. 2. Reversible inhibition of MAO by FBF. Duplicate assays contained 2×10^{-5} M tryptamine and MAO in the range 0·8–4·0 μg protein (●) no inhibitor; (■) 1×10^{-6} M FBF; (▲) 2×10^{-6} M FBF.

TABLE 2. REMOVAL OF FBF INHIBITION BY DIALYSIS

	Fraction	Activity (nmoles/min/mg protein)	% Inhibition
I	Dialysed, no inhibitor	12·2 ± 1·5	
II	Dialysed with inhibitor	10·9 ± 0·8	12
III	Fraction I, assayed with inhibitor	1·8 ± 0·3	86

Fractions II and I, containing 3 ml enzyme samples (8 μg/ml protein), with and without FBF (2×10^{-5} M) respectively, were dialysed at 0–4° for 24 hr against 0·01 M potassium phosphate buffer (41, pH 7·4). 0·5 Ml aliquots of the enzyme fractions were assayed in duplicate with 2×10^{-5} M substrate. FBF concentration in the assay was 7×10^{-6} M. Results show mean and standard error for three similarly treated enzyme samples.

TABLE 3. *In vivo* INHIBITION OF MAO BY FBF

	Control (Coconut Oil)	Furoxanobenzofuroxan 15 mg/kg	50 mg/kg
5-HT heart	0·29 ± 0·02	0·27 ± 0·01	0·45 ± 0·03*
5-HT brain	0·52 ± 0·02	0·65 ± 0·02*	0·93 ± 0·05*
5-HT liver	0·37 ± 0·02	0·34 ± 0·01	0·52 ± 0·03*
NA heart	0·88 ± 0·02	0·77 ± 0·04	1·58 ± 0·12*
NA brain	0·50 ± 0·05	0·43 ± 0·01	0·67 ± 0·05*
NA liver	0·69 ± 0·10	0·61 ± 0·10	0·62 ± 0·10

Guinea-pigs were injected with FBF (0, 15 or 50 mg/kg) in coconut oil. Results show biogenic amine levels 4 hr after injection, in terms of μg amine/g tissue (wet wt), and are given as means and standard errors for groups of six to nine animals.

* Change from control significant at 5 per cent level using the Student *t* test.

TABLE 4. *In vivo* INHIBITION OF MAO BY TRANYLCYPROMINE

	Control (Saline)	Tranylcypromine (10 mg/kg)
5-HT heart	0·25 ± 0·01	0·27 ± 0·02
5-HT brain	0·52 ± 0·04	0·57 ± 0·04
5-HT liver	0·31 ± 0·06	0·32 ± 0·03
NA heart	0·84 ± 0·04	1·09 ± 0·06*
NA brain	0·44 ± 0·02	0·66 ± 0·03*
NA liver	0·50 ± 0·03	0·48 ± 0·08

Guinea-pigs were injected intraperitoneally with saline containing tranylcypromine (0 or 10 mg/kg). Results are expressed as in Table 3.

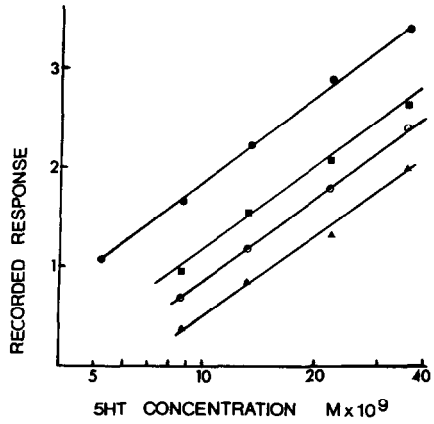


FIG. 3. Inhibition by FBF of 5-HT-induced contractions of the rat fundus strip. The rat fundus strip was set up in a 30 ml organ bath containing de Jalon's solution. Drugs were added to the bath every 2 min using the cumulative method. Contractions of the muscle (expressed in terms of movement of the recorder pen) are plotted against bath concentration of 5-HT (log scale) (●) no FBF; (■) $3{\cdot}4 \times 10^{-8}$ M FBF; (○) $6{\cdot}9 \times 10^{-8}$ M FBF; (▲) $1{\cdot}7 \times 10^{-7}$ M FBF. Each point is the mean of three determinations.

dialysis (Table 2), a further indication that the inhibitor–enzyme interaction was reversible.

The effects of FBF on biogenic amine levels *in vivo* were examined, and the results appear in Tables 3 and 4. The lower dose of FBF produced a significant elevation in brain 5-HT levels, but no changes in NA levels. An equimolar dose of tranylcypromine caused elevation of NA in heart and brain, but no elevation in 5-HT. A higher dose of FBF caused elevation of 5-HT in all three organs examined, while NA levels were elevated in the heart and brain.

Since FBF inhibited MAO in a reversible and partially competitive manner, and showed some specificity in elevating 5-HT *in vivo*, its ability to interact with other 5-HT receptors was examined. Log dose–response curves for 5-HT-induced contrac-

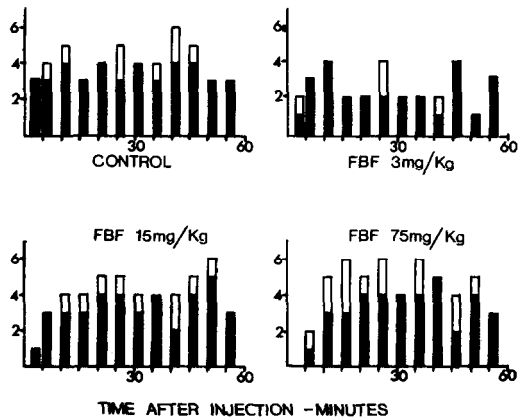


FIG. 4. Response to FBF in the mouse twitch test. Pairs of mice were injected i.p. with FBF in coconut oil, or with coconut oil alone, and observed for a period of 2 min every 5 min. Each treatment group contained a total of ten mice. Results show the number of mice showing a response in the observation period (shaded area) and the total number of responses observed during this time (total area).

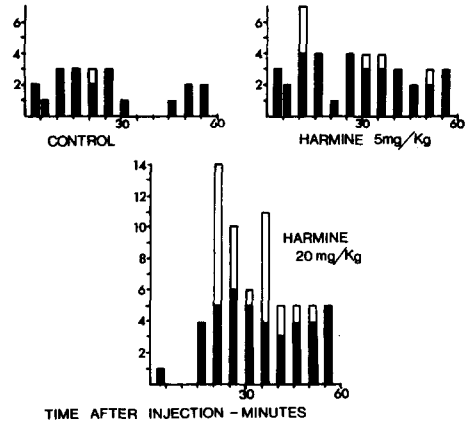


FIG. 5. Response to harmine in the mouse twitch test. Pairs of mice were injected i.p. with harmine in isotonic saline, or with saline alone. Animals were observed and results expressed as described for Fig. 4.

tions of the isolated rat fundus strip are shown in Fig. 3. Marked reduction in the response to 5-HT (5×10^{-9}–3×10^{-8} M) was produced by FBF (10^{-7}–10^{-9} M). (FBF caused small dose-dependent relaxations of the muscle. Agonists were added 3 min after the introduction of FBF to the bath, when a stable base line had been reached). Regression analysis of the log dose–response lines showed them to be parallel, indicating that the drug and 5-HT were influencing the same receptor. There was no reduction in the responses to either barium ions (8×10^{-5}–5×10^{-4} M) or acetylcholine (2×10^{-9}–3×10^{-8} M) at these concentrations of FBF.

The action of FBF in blocking the action of 5-HT peripherally while elevating its levels centrally was similar to that reported for the harmala alkaloids, which have hallucinogenic activity in man.[3] Possible hallucinogenic effects of FBF and harmine were compared using the mouse twitch test.[10] Lysergic acid diethylamide (LSD) was used as a reference compound. Figures 4 and 5 show the frequency of twitch responses observed in this test for FBF and harmine respectively. These may be compared with the response to LSD (Fig. 6). With FBF there was no apparent change

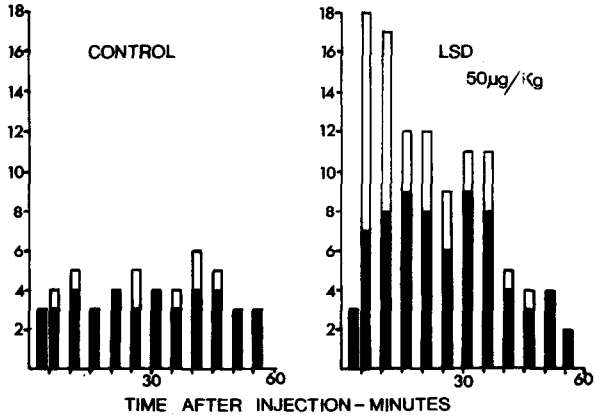


FIG. 6. Response to LSD in the mouse twitch test. Pairs of mice were injected i.p. with LSD in coconut oil, or with coconut oil alone. Animals were observed and results expressed as described for Fig. 4.

in the response frequency at the three dose levels tested. The group injected with 15 mg/kg, a dose sufficient to elevate levels of 5-HT in guinea-pig brain in 4 hr, was observed for a total of 4 hr but no difference in response from the control group was evident during this time.

Because the coconut oil control group showed an increase in response frequency over the saline control group (a total of 49 responses recorded over the entire observation period vs 21 for the saline), FBF (15 mg/kg) was also tested as a suspension in 0·5% methyl cellulose. The control group in this experiment gave a lower response (total of 30 responses) while the drug group showed 27 responses, confirming the absence of activity of FBF in this test.

The animals injected with harmine appeared to show an increase in response frequency over their corresponding control group (Fig. 5). The increased response in the 20 mg/kg group was only evident after 20 min, when the characteristic tremor produced by the drug had disappeared. It appears from these results that an increase in response was produced by harmine, while FBF was ineffective. However, the response to harmine was small when compared with the results obtained for LSD (Fig. 6).

Peripheral blockers of 5-HT (e.g. methysergide) have been shown to prevent the production of head twitching by drugs with 5-HT-like activity e.g. 5-hydroxytryptophan (5-HTP) LSD and mescaline.[10] FBF was tested for central 5-HT-blocking activity by its ability to reduce the twitch response to 5-HTP. The results are shown in Fig. 7. The group injected with 5-HTP (100 mg/kg) showed a marked increase in response frequency over the controls, and this increase was partially, but not completely, prevented by FBF (15 mg/kg).

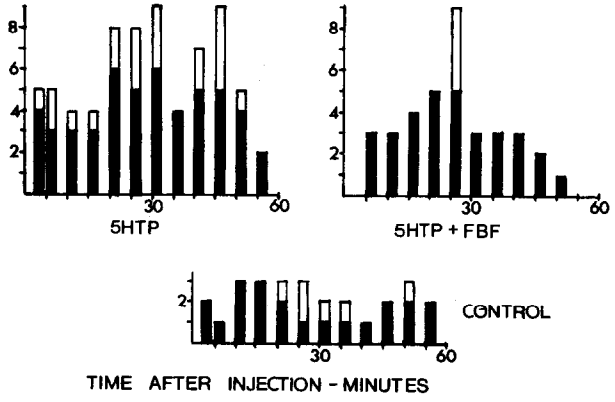


FIG. 7. Effect of FBF on the response to 5-hydroxytryptophan in the mouse twitch test. The control group was injected i.p. with 0·1 ml coconut oil per 20 g mouse, followed after 30 min by 0·15 ml/20 g mouse of isotonic saline. The 5-HTP group received coconut oil followed by 100 mg/kg 5-HTP and the 5-HTP/FBF group received 15 mg/kg FBF in coconut oil followed after 30 min by 100 mg/kg 5-HTP in isotonic saline. Observation, carried out as described for Fig. 4, was begun after the second injection. Results are expressed as described for Fig. 4.

DISCUSSION

Monoamine oxidase appears to consist, not of a single entity, but of two enzymes or groups of enzymes differing in substrate specificity.[13,14] Fuller[15] demonstrated selectivity of inhibitors *in vitro* by comparing I_{50} values obtained using different substrates. Harmaline and Lilly 51641 were more effective in preventing oxidation of indolealkylamine substrates, while pargyline and tranylcypromine were more potent when phenylethylamine substrates were used. From the difference in specificity demonstrated between FBF and tranylcypromine *in vivo*, FBF appears to belong to the former class of inhibitors.

Differential inhibition of MAO species has also been demonstrated *in vitro* by Squires[13] and Johnston.[11] Squires[13] showed that a mouse mitochondrial preparation was inhibited biphasically by harmine and pargyline, with enzymic activity which was relatively harmine sensitive and pargyline resistant, as well as activity which was harmine resistant and pargyline sensitive. The relative proportions of these activities varied with the organ from which the enzyme was prepared. A similar biphasic MAO inhibition was demonstrated for M & B 9302 by Johnston[11] who found that the enzyme species preferentially inhibited appeared to be responsible for the oxidation of 5-HT and other indolealkylamines.

Despite the apparent inhibition specificity found for FBF *in vivo*, no evidence was found for biphasic inhibition by FBF *in vitro*, although almost complete inhibition was achieved. The curve showing increasing inhibition had a simple hyperbolic shape, in contrast to the biphasic plots described above, and the sigmoid inhibition plot found for iproniazid.[11]

The partially competitive, reversible inhibition of MAO by FBF, as well as the apparent specificity for indolealkylamine substrates *in vivo*, indicated that a structural similarity to 5-HT might be responsible for the actions of FBF. In fact, when FBF was tested at peripheral 5-HT receptors, it was a powerful antagonist to the actions of 5-HT, and itself caused muscle relaxation, perhaps due to interference with the action of endogenous 5-HT. Thus the structural similarity to 5-HT was sufficient to allow FBF to bind, but not to stimulate these receptors.

In contrast, the harmala alkaloids have been reported to exert some peripheral effects similar to those of 5-HT, as well as 5-HT antagonism in animals. Unlike harmine, FBF was inhibitory rather than stimulatory in the mouse twitch test, indicating that centrally, as well as peripherally, its 5-HT blocking activity predominated over any 5-HT-like effects due to elevation in levels of 5-HT.

As an MAO inhibitor, FBF appears to rank with the recently described compounds M & B 9302[15] and Lilly 51641,[14] differing in specificity from the clinically used inhibitors pargyline and tranylcypromine. FBF thus appears to have two potentially useful activities. As a 5-HT antagonist, it could find a place with such compounds as methysergide, which has proved valuable in the treatment of migraine. As an MAO inhibitor for use in depression, it is of interest both because of its preferential action in the brain and because of its specificity in affecting levels of indolealkylamines. Such an MAO inhibitor should be free not only from peripheral side-effects but also from effects due to interaction with exogenous phenylethylamines e.g. tyramine in food. This compound therefore should provide a useful starting point for the synthesis of molecules retaining the potency and specificity of FBF while separating its 5-HT antagonist and MAO inhibitory effects.

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