J. Pharm. Pharmacol. 1987, 39: 1003–1009
Received July 3, 1987



# Biochemical and pharmacological tests for the prediction of ability of monoamine uptake blockers to inhibit the uptake of noradrenaline in-vivo: the effects of desipramine, maprotiline, femoxetine and citalopram*

LESZEK PAWŁOWSKI† AND GABRIEL NOWAK

*Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Kraków, Poland*

The ability of desipramine and maprotiline (NA uptake inhibitors), as well as citalopram and femoxetine (5-HT uptake inhibitors) to protect mice against brain NA depletion induced by H 77/77 (4-α-dimethyl-*m*-tyramine), has been compared with their ability to counteract reserpine (2·5 mg $kg^{-1}$)- or apomorphine (16 mg $kg^{-1}$)-induced hypothermia and to potentiate TRH (40 mg $kg^{-1}$)-induced hyperthermia in mice. While both NA uptake inhibitors antagonized the action of H 77/77, maprotiline being weaker than desipramine, femoxetine and citalopram were inactive. However, in contrast to citalopram, femoxetine was active in the other tests, being about twice as weak as maprotiline, which itself was several times weaker than desipramine in those tests. On the basis of the results obtained it is concluded that functional in-vivo tests for NA uptake inhibitors are more sensitive than the H 77/77 biochemical test; moreover, femoxetine, which in in-vitro studies is less selective than citalopram, may inhibit the uptake of NA in-vivo.

Most antidepressant drugs are noradrenaline (NA)-uptake inhibitors (Richelson & Pfenning 1984), some (e.g. desipramine, maprotiline, oxaprotiline, protriptyline) being extremely potent and/or highly selective (Maître et al 1980; Hyttel 1982; Richelson & Pfenning 1984). Therefore the view that the antidepressant effect can be achieved by inhibition of NA uptake (Glowinski & Axelrod 1964) has become widely accepted. However, among the new potential antidepressants that have been, or are being, tested in the clinic, are drugs such as zimelidine (Åberg & Holmberg 1979; Coppen et al 1979; Montgomery 1980; Åberg 1981; Montgomery et al 1981), femoxetine (Ghose et al 1977; Bøjholm et al 1979; Reebye et al 1982), cianopramine (Hormazabal et al 1985) and citalopram (Gottlieb et al 1980; Øfsti 1982), which are potent and relatively selective inhibitors of the uptake of 5-hydroxytryptamine (5-HT) (for comparison with other drugs of this type see: Maître et al 1980; Hyttel 1982). These drugs have been developed as a consequence of the 5-HT theory of depression, put forward by Coppen (1967), Lapin & Oxenkrug (1969) and van Praag (1974) after the discovery that imipramine, besides its known effect on the NA uptake (Glowinski & Axelrod 1964), also inhibited the uptake of 5-HT. The clinical efficacy of these 'selective' drugs (more selective than clomipramine) remains to be proven (see: Ghose et al 1977; Maître et al 1980); up to the present only the efficacy of zimelidine has been sufficiently documented (Maître et al 1980), but it has failed because of its potential to cause neurological disorders. From the theoretical point of view (see above), it must also be established whether these drugs (including their metabolites) are actually devoid of any effect on the uptake of NA. Clomipramine, which for several years had been considered a fairly selective 5-HT uptake inhibitor (on the basis of the in-vitro results), and an antidepressant which acts exclusively through the 5-HT system, only recently has been recognized as a relatively potent NA uptake inhibitor under in-vivo conditions (Maj et al 1982; Ögren et al 1983; Pawłowski & Kwiatek 1983a, b). As has recently become known, its main metabolite, desmethylclomipramine, which accumulates in the body of patients treated with clomipramine (Jones & Luscombe 1977; Träskman et al 1979), very potently inhibits the uptake of NA in-vivo and in-vitro (Hyttel 1982; Maj et al 1982; Pawłowski & Kwiatek 1983b).

We have previously shown (Pawłowski & Mazela 1984; Pawłowski et al 1984, 1985) that some of the new 'specific' 5-HT uptake inhibitors (e.g., cianopramine) or, at least, their *N*-desmethyl metabolites

* The results were presented at the 6th International Catecholamine Symposium, Jerusalem, June 14–19, 1987.

† Correspondence.

(e.g., desmethylcianopramine, norzimelidine) behaved as inhibitors of NA uptake in a series of appropriate pharmacological tests, such as reserpine hypothermia in mice (Slater et al 1979; Maj et al 1982, 1983; Przegaliński et al 1983), apomorphine hypothermia in mice (Puech et al 1981; Pawłowski & Mazela 1986) and TRH hyperthermia in mice (Desiles & Rips 1981; Pawłowski & Kwiatek 1983a, b). Moreover, we found that cianopramine and desmethylcianopramine could protect rat and mouse brain against NA depletion caused by H 77/77 (Pawłowski et al 1984, 1985). Such a protection is also characteristic of NA uptake inhibitors (Carlsson et al 1969; Maître et al 1980). Thus, the results of our studies suggest that those antidepressants previously regarded as selective 5-HT uptake inhibitors on the basis of in-vitro data, may, like clomipramine, also influence NA uptake in-vivo. At the same time, our results (Pawłowski et al 1984, 1985) have suggested that the above simple pharmacological tests (Slater et al 1979; Desiles & Rips 1981; Puech et al 1981; Maj et al 1982, 1983; Pawłowski & Kwiatek 1983a, b; Przegaliński et al 1983; Pawłowski & Mazela 1986), initially used in the screening of potential antidepressant agents, are much more sensitive as indicators of a drug's ability to inhibit the uptake of NA in-vivo than is the classical biochemical test using H 77/77 (Carlsson et al 1969; Maître et al 1980).

To find whether this suggestion is true, we have compared the effects of four monoamine uptake blockers (desipramine, maprotiline, femoxetine, citalopram) in the H 77/77 test and in pharmacological tests for NA uptake inhibitors. Among the drugs tested, desipramine is regarded as an extremely potent and highly selective NA uptake inhibitor, maprotiline as a moderately potent but extremely selective NA uptake inhibitor, femoxetine as a potent 5-HT uptake inhibitor, more selective than clomipramine, and citalopram is rated as an extremely selective and potent 5-HT uptake inhibitor (Ögren et al 1983). The ability of these four drugs, and other related compounds, to inhibit the uptake of NA and 5-HT in-vitro is shown in Table 1, which presents the data obtained in the same laboratory by Hyttel (1982) and Hyttel & Larsen (1985).

## MATERIALS AND METHODS

### *Animals*

Male Albino-Swiss mice (18–34 g) were kept in colony cages with free access to food (granular standard diet, Bacutil) and tap water until the beginning of the experiment.

### *Drugs*

The drugs were used: apomorphine hydrochloride (Sandoz), citalopram hydrobromide (Lundbeck), desipramine hydrochloride (Pertofran; Ciba Geigy), femoxetine hydrochloride (FG 4963; Ferrosan), H 77/77 (4,α-dimethyl-*m*-tyramine methylester hydrochloride; Astra), maprotiline hydrochloride (Ludiomil; Ciba Geigy), norfemoxetine hydrochloride (Ferrosan), norzimelidine dihydrochloride (Astra), reserpine (Rausedyl, amp.; Gedeon Richter), TRH (thyrotropin releasing hormone, pyroglutamyl-histydyl-prolinamide; Scientific Research Division of the Institute of Chemistry of the University of Gdańsk), zimelidine dihydrochloride (Astra). Doses refer to the salts given. The drugs were administered intraperitoneally (i.p.) or subcutaneously (s.c.) in a volume of 10 mL $kg^{-1}$. Except for apomorphine, H 77/77 and TRH, which were dissolved in 0·9% NaCl (saline), all the other drugs were dissolved in redistilled water. To avoid the oxidation of apomorphine, its solution was always prepared freshly, i.e. not later than 5–10 min before the administration.

### *H 77/77-induced depletion of brain NA*

Mice were injected twice with H 77/77, 12·5 mg $kg^{-1}$ i.p., the doses being administered 2 h apart. The test drugs or the solvent were administered i.p. 30 min before each dose of H 77/77, the second dose of the test drugs being half the amount of the first dose. The animals were decapitated 2 h after the last dose of H 77/77 and whole brains were immediately removed and frozen until assay. The brains were homogenized in cold 0·4 M perchloric acid. After centrifugation, NA was extracted according to Earley & Leonard (1978) and then determined fluorometrically according to Chang (1964). Each experimental group consisted of six to seven animals. The statistical significance of the results was assessed by Student's *t*-test (two-tailed). The percentage inhibition of NA displacement induced by H 77/77 was calculated using the formula of Bruinvels (1971).

### *Reserpine- and apomorphine-induced hypothermia and TRH-induced hyperthermia*

Before the experiment the mice were allowed to adapt for 24 h to the conditions (temperature of 20 ± 1·5 °C for reserpine-induced hypothermia, 21 ± 1 °C for apomorphine-induced hypothermia or 22 ± 1 °C for TRH-induced hyperthermia). Rectal temperature was measured with an Ellab T-3 thermometer at times specified in the Tables. The results are expressed as a change in body temperature (ΔT)

Table 1. Effects of established and potential antidepressant drugs, and some of their metabolites on the uptake of [$^3$H]amines into rat brain synaptosomes according to Hyttel (1982) and Hyttel & Larsen (1985). The results are expressed as IC50 values in nM.

| Drug (IC50 values in nM) | NA | 5-HT | NA/5-HT |
|---|---|---|---|
| Imipramine* | 20 | 35 | 0·57 |
| Desipramine* | 0·97 | 210 | 0·0046 |
| Maprotiline* | 8·4 | 3000 | 0·0028 |
| Clomipramine*, ** | 24 | 1·5 | 16 |
| Desmethylclomipramine*, ** | 0·46 | 41 | 0·011 |
| Cianopramine*, ** | 46 | 0·87 | 53 |
| Desmethylcianopramine** | 1·4 | 15 | 0·093 |
| Femoxetine*, ** | 410 | 8·3 | 49 |
| Norfemoxetine** | 410 | 2·1 | 195 |
| Zimelidine*, ** | 3200 | 59 | 54 |
| Norzimelidine*, ** | 260 | 9·6 | 27 |
| Citalopram*, ** | 8800 | 1·8 | 4889 |
| Desmethylcitalopram*, ** | 780 | 7·4 | 105 |
| Didesmethylcitalopram*, ** | 1500 | 24 | 63 |
| Citalopram-*N*-oxide*, ** | 3200 | 56 | 57 |

* Results from Hyttel (1982).
** Results from Hyttel & Larsen (1985).

with respect to the initial rectal temperature measured immediately before the injection of the test drugs. These were administered i.p., 20 h after s.c. injection of reserpine (2·5 mg kg$^{-1}$) or 30 min before injection of apomorphine (16 mg kg$^{-1}$ s.c.) or TRH (40 mg kg$^{-1}$ i.p.). Each group consisted of 5–12 mice. The statistical significance of results was assessed by Student's *t*-test (two-tailed).

## RESULTS

### *H 77/77-induced depletion of brain NA*

The effects of the monoamine uptake inhibitors (desipramine, maprotiline, citalopram, zimelidine, femoxetine) and some of their *N*-desmethyl metabolites (norzimelidine, norfemoxetine) on H 77/77-induced brain NA displacement are shown in Table 2. Desipramine potently antagonized the brain NA depletion induced by H 77/77, and its potency (the range of effective doses) was almost exactly the same as that reported earlier by Carlsson et al (1969). Also maprotiline dose-dependently antagonized the depletion, but its active doses (producing more than a 10% inhibition of the effect of H 77/77) were much higher than those of desipramine. Thus, the lowest dose of maprotiline which produced a statistically significant inhibition of the effect of H 77/77 was as high as 40 + 20 mg kg$^{-1}$ (127·3 + 63·6 μmol kg$^{-1}$); an analogous dose for desipramine was 5 + 2·5 mg kg$^{-1}$ (17·3 + 8·6 μmol kg$^{-1}$). The other drugs, used in doses up to 40 + 20 mg kg$^{-1}$ (99–121·5 + 49·5–60·7 μmol kg$^{-1}$), were inactive in the test; the higher doses, i.e. 80 + 40 and 160 + 80 mg kg$^{-1}$, were not tested, since in a preliminary experiment they caused a 40–100% mortality.

### *Reserpine- and apomorphine-induced hypothermia and TRH-induced hyperthermia*

At the doses used, the monoamine uptake inhibitors (desipramine, maprotiline, citalopram, femoxetine) either did not affect body temperature in mice or decreased it (data not shown).

Of the four drugs, desipramine (0·3125–1·25 mg kg$^{-1}$; 1–4·3 μmol kg$^{-1}$), maprotiline (1·25–5 mg kg$^{-1}$; 3·9–15·9 μmol kg$^{-1}$ and femoxetine (2·5–10 mg kg$^{-1}$; 7·2–29·1 μmol kg$^{-1}$) were active in the all three tests, having antagonized the reserpine (2·5 mg kg$^{-1}$)- and apomorphine (16 mg kg$^{-1}$)-induced hypothermia, and having potentiated the TRH (40 mg kg$^{-1}$)-induced hyperthermia in a dose-dependent manner. The order of potencies was as follows: desipramine ≫ maprotiline > femoxetine (see Tables 3–5). In contrast, citalopram (2·5–40 mg kg$^{-1}$; 6·1–99 μmol kg$^{-1}$) was inactive in all of these tests (Tables 3–5).

None of the active drugs, nor citalopram, antagonized hypothermia induced by 1 mg kg$^{-1}$ of apomorphine which, according to Puech et al (1981) and Pawłowski & Mazela (1986), is antagonized easily by dopamine receptor blockers but not my monoamine (NA) uptake inhibitors (data not shown).

## DISCUSSION

The present results confirm and extend our earlier findings (Pawłowski et al 1984, 1985) that the pharmacological (functional) in-vivo tests for NA

Table 2. Effects of monoamine uptake inhibitors and some of their *N*-desmethyl metabolites (norzimelidine, norfemoxetine) on the displacement of the brain noradrenaline (NA) by 4α-dimethyl-*m*-tyramine (H 77/77) in mice.

| Treatment | First drug dose (mg kg$^{-1}$ i.p.) | Drug alone | Drug + H 77/77 | % Inhibition |
|---|---|---|---|---|
| | | Brain NA (ng g$^{-1}$ ± s.e.m.) | | |
| Solvent | — | 393 ± 21 | 193 ± 8**** | — |
| Desipramine | 2·5 | 398 ± 18 | 211 ± 14 | 8 |
| Desipramine | 5 | 385 ± 20 | 233 ± 12* | 22 |
| Desipramine | 10 | 352 ± 24 | 244 ± 14* | 40 |
| Maprotiline | 10 | 385 ± 45 | 199 ± 10 | 5 |
| Maprotiline | 20 | 366 ± 15 | 218 ± 17 | 21 |
| Maprotiline | 40 | 352 ± 20 | 248 ± 11** | 42 |
| Citalopram | 40 | 330 ± 23 | 189 ± 27 | 0 |
| Zimelidine | 40 | 366 ± 22 | 197 ± 16 | 0 |
| Norzimelidine | 40 | 392 ± 23 | 193 ± 25 | 0 |
| Femoxetine | 40 | 369 ± 14 | 197 ± 8 | 0 |
| Norfemoxetine | 40 | 343 ± 14 | 197 ± 12 | 0 |

**** $P < 0.001$ when compared with solvent value; ** $P < 0·01$; * $P < 0·02$ when compared with solvent + H 77/77 value.

Mice were injected with two doses of H 77/77 (12·5 mg kg$^{-1}$ i.p.) 2 h apart. All animals were killed 2 h after the last dose of H 77/77. Test drugs were administered 30 min before each dose of H 77/77, the second dose of drug being half the first. Each result is the mean ± s.e.m. of 6–7 mice. The statistical significance of the results was assessed by Student's *t*-test.

uptake inhibitors are much more sensitive than the biochemical (H 77/77) test originally introduced by Carlsson et al (1969) and recommended by Maître et al (1980) as a test of choice for the in-vivo screening of monoamine uptake blockers. Although the classical NA uptake inhibitor desipramine was very active in the H 77/77 test, since a dose of 5 + 2·5 mg kg$^{-1}$ significantly protected the mice against brain NA depletion caused by the agent, the drug was more active in the pharmacological tests (Tables 3–5). Maprotiline, which in-vitro is a NA uptake inhibitor about 8–9 times weaker than desipramine (see Table 1), was also proportionally weaker in the H 77/77 test, but its lowest effective dose (i.e. 40 + 20 mg kg$^{-1}$) in that test was only twice as low as the lethal one (i.e. 80 + 40 mg kg$^{-1}$) which produced death in about 50% of animals. On the other hand, in pharmacological tests, maprotiline was active in its normal dose range, i.e. 2·5–20 mg kg$^{-1}$ (see Tables 3–5).

In view of these findings, a question arises whether negative results from the H 77/77 test necessarily mean that the compound tested is devoid of an

Table 3. Effects of monoamine uptake inhibitors upon reserpine (2·5 mg kg$^{-1}$)-induced hypothermia in mice kept at the ambient temperature of 20 ± 1·5 °C.

| Compound | mg kg$^{-1}$[a] | Inital temperature °C[b] | Δt (°C ± s.e.m.) after 60 min | 120 min | 180 min | 240 min |
|---|---|---|---|---|---|---|
| Solvent | | 22·4 ± 0·45 | 1·7 ± 0·52 | 2·5 ± 0·68 | 3·8 ± 0·80 | 4·8 ± 0·88 |
| Desipramine | 1·25 | 22·8 ± 0·51 | 4·9 ± 0·57*** | 8·8 ± 0·45*** | 9·3 ± 0·42*** | 9·1 ± 0·40*** |
| Solvent | | 21·4 ± 0·24 | 2·3 ± 0·33 | 3·3 ± 0·61 | 4·5 ± 0·68 | 5·3 ± 0·71 |
| Maprotiline | 1·25 | 22·3 ± 0·66 | 1·9 ± 0·35 | 3·7 ± 0·57 | 6·5 ± 0·85 | 7·5 ± 0·90 |
| Maprotiline | 2·5 | 21·8 ± 0·44 | 3·4 ± 0·64 | 5·4 ± 0·55 | 6·6 ± 0·60* | 6·9 ± 0·54 |
| Maprotiline | 5 | 21·8 ± 0·44 | 3·9 ± 0·82 | 7·0 ± 0·92** | 8·1 ± 0·78** | 8·5 ± 0·52*** |
| Solvent | | 24·1 ± 0·91 | 2·4 ± 0·60 | 4·0 ± 0·90 | 5·5 ± 0·92 | 6·1 ± 0·95 |
| Femoxetine | 5 | 22·8 ± 0·53 | 2·5 ± 0·39 | 5·1 ± 0·87 | 6·4 ± 0·90 | 7·0 ± 0·98 |
| Femoxetine | 10 | 22·9 ± 0·87 | 4·8 ± 0·79* | 8·0 ± 0·90** | 9·1 ± 0·72** | 8·2 ± 0·68 |
| Solvent | | 24·6 ± 1·28 | 2·5 ± 1·11 | 3·0 ± 1·19 | 2·7 ± 1·13 | 2·6 ± 1·30 |
| Citalopram | 2·5 | 28·0 ± 1·25 | −0·3 ± 1·02 | 0·0 ± 1·52 | −0·5 ± 1·74 | 1·3 ± 1·75 |
| Citalopram | 10 | 22·3 ± 0·90 | 3·0 ± 0·97 | 3·7 ± 1·04 | 3·2 ± 1·04 | 2·6 ± 1·16 |
| Citalopram | 40 | 26·0 ± 1·48 | 0·6 ± 1·24 | 0·5 ± 1·29 | 0·5 ± 1·31 | 0·2 ± 1·41 |

[a] The drugs were administered 20 h after the reserpine injection.

[b] The rectal temperature of the mice before the reserpine injection was 36·8–38·4 °C. Each experimental group consisted of 8 (citalopram) or 10–12 (desipramine, maprotiline, femoxetine) mice.

* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$ (difference from the control/solvent/group; Student's *t*-test).

Table 4. Effects of desipramine (DMI), maprotiline (MAP), femoxetine (FEM) and citalopram (CIT) on apomorphine (APO; 16 mg kg$^{-1}$)-induced hypothermia in mice kept at the ambient temperature of 21 ± 1 °C.

| Treatment mg kg$^{-1}$[a] | | | Change from baseline rectal temperature (ΔT) at x min after challenge (°C ± s.e.m.) x = 30 | x = 45 | x = 60 |
|---|---|---|---|---|---|
| | | APO 16 | −4·3 ± 0·66 | −4·8 ± 0·58 | −4·4 ± 0·35 |
| DMI | 0·3125 | + APO 16 | −3·4 ± 0·51 | −3·9 ± 0·70 | −4·2 ± 0·53 |
| DMI | 0·625 | + APO 16 | −2·2 ± 0·59* | −2·9 ± 0·64 | −3·4 ± 0·58 |
| DMI | 1·25 | + APO 16 | −1·5 ± 0·60* | −1·4 ± 0·52** | −1·5 ± 0·52** |
| | | APO 16 | −4·7 ± 0·35 | −4·5 ± 0·44 | −4·1 ± 0·53 |
| MAP | 1·25 | + APO 16 | −4·0 ± 0·47 | −3·8 ± 0·50 | −3·6 ± 0·51 |
| MAP | 2·5 | + APO 16 | −3·6 ± 0·40 | −3·5 ± 0·44 | −3·4 ± 0·45 |
| MAP | 5 | + APO 16 | −2·6 ± 0·46** | −2·8 ± 0·50* | −3·1 ± 0·32 |
| | | APO 16 | −5·0 ± 0·49 | −5·1 ± 0·55 | −4·3 ± 0·61 |
| FEM | 2·5 | + APO 16 | −4·3 ± 0·35 | −4·4 ± 0·37 | −3·7 ± 0·51 |
| FEM | 5 | + APO 16 | −4·2 ± 0·47 | −4·6 ± 0·46 | −4·2 ± 0·42 |
| FEM | 10 | + APO 16 | −3·0 ± 0·52* | −3·6 ± 0·49 | −4·2 ± 0·44 |
| | | APO 16 | −5·1 ± 0·49 | −5·8 ± 0·55 | −5·8 ± 0·57 |
| CIT | 2·5 | + APO 16 | −4·2 ± 0·45 | −4·7 ± 0·44 | −4·4 ± 0·47 |
| CIT | 10 | + APO 16 | −5·2 ± 0·33 | −5·5 ± 0·37 | −4·6 ± 0·41 |
| CIT | 40 | + APO 16 | −5·9 ± 0·51 | −5·6 ± 0·53 | −5·2 ± 0·37 |

[a] The drugs (or solvent) were injected 30 min before apomorphine. Each experimental group consisted of 5 (desipramine) to 10 (maprotiline, femoxetine, citalopram) mice.
* $P < 0·05$; ** $P < 0·01$ (difference from the respective control group receiving apomorphine alone; Student's $t$-test).

inhibitory effect on the uptake of NA in-vivo. Our present results clearly suggest that in some instances biochemical in-vivo tests (e.g. the H 77/77 test) may yield false negative results, since they are not sensitive enough to detect a negligible inhibitory effect on NA uptake. Of the monoamine uptake blockers tested by us in this and previous (Pawłowski & Mazela 1984; Pawłowski et al 1984, 1985) studies, only citalopram may be regarded as completely devoid of any effect on the uptake of NA in-vitro and in-vivo. It affects the uptake of NA in-vitro exclusively at extremely high, 'non-physiological' concentrations, and its main metabolites (desmethylcitalopram, didesmethylcitalopram and citalopram-*N*-

Table 5. Effects of desipramine (DMI), maprotiline (MAP), femoxetine (FEM) and citalopram (CIT) on TRH (40 mg kg$^{-1}$)-induced hyperthermia in mice kept at the ambient temperature of 22 ± 1 °C.

| Treatment mg kg$^{-1}$[a] | | | Change from baseline rectal temperature (ΔT) at x min after challenge (°C ± s.e.m.) x = 15 | x = 30 | x = 45 | x = 60 |
|---|---|---|---|---|---|---|
| | | TRH 40 | 1·1 ± 0·20 | 0·8 ± 0·24 | 0·5 ± 0·22 | 0·3 ± 0·29 |
| DMI | 0·3125 | + TRH 40 | 2·3 ± 0·25** | 0·6 ± 0·29 | 0·3 ± 0·20 | −0·1 ± 0·21 |
| DMI | 0·625 | + TRH 40 | 2·8 ± 0·19*** | 0·8 ± 0·29 | 0·1 ± 0·22 | −0·4 ± 0·33 |
| DMI | 1·25 | + TRH 40 | 3·0 ± 0·20*** | 1·1 ± 0·27 | 0·6 ± 0·27 | 0·2 ± 0·33 |
| | | TRH 40 | 0·8 ± 0·14 | 0·8 ± 0·23 | 0·6 ± 0·23 | 0·6 ± 0·22 |
| MAP | 1·25 | + TRH 40 | 1·2 ± 0·22 | 1·0 ± 0·29 | 0·7 ± 0·28 | 0·5 ± 0·26 |
| MAP | 2·5 | + TRH 40 | 1·7 ± 0·22** | 0·8 ± 0·30 | 0·4 ± 0·36 | 0·1 ± 0·27 |
| MAP | 5 | + TRH 40 | 2·4 ± 0·25*** | 1·4 ± 0·22 | 0·9 ± 0·29 | 0·7 ± 0·25 |
| | | TRH 40 | 1·2 ± 0·29 | 1·2 ± 0·20 | 1·1 ± 0·16 | 0·9 ± 0·16 |
| FEM | 2·5 | + TRH 40 | 1·6 ± 0·44 | 1·6 ± 0·30 | 1·3 ± 0·23 | 0·9 ± 0·18 |
| FEM | 5 | + TRH 40 | 2·0 ± 0·36 | 1·7 ± 0·23 | 1·4 ± 0·17 | 1·0 ± 0·15 |
| FEM | 10 | + TRH 40 | 2·4 ± 0·53* | 2·0 ± 0·35 | 1·7 ± 0·40 | 1·2 ± 0·33 |
| | | TRH 40 | 0·9 ± 0·22 | 0·9 ± 0·24 | 0·7 ± 0·25 | 0·6 ± 0·27 |
| CIT | 2·5 | + TRH 40 | 0·6 ± 0·26 | 0·7 ± 0·13 | 0·6 ± 0·11 | 0·5 ± 0·13 |
| CIT | 10 | + TRH 40 | 1·2 ± 0·25 | 0·9 ± 0·32 | 0·8 ± 0·28 | 0·7 ± 0·20 |
| CIT | 40 | + TRH 40 | 1·4 ± 0·27 | 1·0 ± 0·26 | 0·5 ± 0·18 | 0·3 ± 0·09 |

[a] The drugs (or solvent) were injected 30 min before TRH. Each experimental group consisted of 8–12 mice.
* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$ (difference from the respective control group receiving TRH alone; Student's $t$-test).

oxide) do not differ from the parent drug in this respect (see Table 1). In addition, citalopram is devoid of any effect on postsynaptic NA receptors (Hyttel 1982; Hall et al 1984). As a consequence, citalopram yields negative results in all the tests used to evaluate NA uptake inhibitors in-vivo (Maître et al 1980; Hyttel 1982; Ögren et al 1983; Pawłowski & Kwiatek 1983a, b; Pawłowski & Mazela 1984, 1986; Pawłowski et al 1984, 1985; results of this study). The selectivity of the other 5-HT uptake inhibitors seems questionable: femoxetine, norzimelidine (an active metabolite of zimelidine) and cianopramine inhibit the uptake of NA in-vitro 20–190 times more strongly than citalopram, however, they are still much weaker (6–50 times) than maprotiline in this respect (Table 1). Therefore, if these three drugs were active in the H 77/77 test, their effective doses might be expected to be proportionally (see the results obtained with desipramine and maprotiline) at least 5–7 times as high as the lowest effective doses of maprotiline; but such high doses of monoamine uptake blockers immediately produce a 100% mortality (Pawłowski, unpublished data). Cianopramine may be exceptionally effective in this test (its lowest effective dose is 20 + 10 mg $kg^{-1}$; Pawłowski et al 1985), since its main metabolite desmethylcianopramine inhibits the uptake of NA more potently than maprotiline (Table 1). Actually, when we investigated norzimelidine and femoxetine in the H 77/77 test—using doses up to 40 + 20 mg $kg^{-1}$ (higher doses were toxic), we did not observe any protective effect against the NA depletion. However, in the pharmacological tests for NA uptake inhibitors (for literature see Introduction and Pawłowski & Mazela 1984) both these drugs were active, being about twice as weak as maprotiline (Pawłowski & Mazela 1984; Tables 3–5).

On the basis of the data in Table 1, we find it somewhat surprising that drugs such as femoxetine or norzimelidine display a relatively potent activity in the functional tests for NA uptake inhibitors, in comparison with maprotiline. This apparent paradox becomes clearer when the postsynaptic action of these drugs is taken into account. As shown by Hall & Ögren (1981), maprotiline and several other drugs of this type (imipramine, desipramine, clomipramine, amitriptyline, nortriptyline), display some affinity for central NA receptors, being weak blockers of these receptors. Femoxetine and norzimelidine (as well as citalopram) are devoid of such effects (Hall & Ögren 1981; Hall et al 1984). Hence, in contrast to maprotiline, femoxetine and norzimelidine are not able to counteract—via NA receptor blockade—their own effects connected with the ability to inhibit the uptake of NA. As was demonstrated earlier (Pawłowski & Kwiatek 1983a, b; Przegaliński et al 1983; Pawłowski & Mazela 1986), functional NA receptors are essential for positive results in the pharmacological tests for NA uptake inhibitors.

It may also be argued that some other factors, different from inhibition of NA uptake, are responsible for the action of norzimelidine and femoxetine in the functional tests. One of the factors in question might be the potential to inhibit monoamine oxidase, the other being the ability to inhibit phosphodiesterase (for literature see Pawłowski & Mazela 1984). However, to our knowledge, neither norzimelidine nor femoxetine (or its desmethyl metabolite norfemoxetine) inhibit the activity of the above-mentioned enzymes in-vitro. Besides, the drugs that inhibit monoamine oxidase yield, in consequence of enzyme inhibition, false positive results in the H 77/77 test (Waldmeier et al 1985), while phosphodiesterase inhibitors do not antagonize apomorphine-induced hypothermia, although they do counteract this induced by reserpine (Przegaliński & Bigajska 1983). Thus, as we have found that norzimelidine and femoxetine are active in the apomorphine hypothermia test, and inactive in the H 77/77 test, their inhibitory effect in-vivo on at least one of the enzymes seems to be extremely unlikely.

In conclusion, the data presented here provided further support for the findings that the results obtained in the H 77/77 test correspond well with the results obtained in-vitro as already reported by Maître et al (1980). However, they also reveal some limitations of this test and, at the same time, show the functional tests as being more sensitive indicators of a drug's ability to inhibit uptake of NA under in-vivo conditions. By means of these tests it was possible to demonstrate the noradrenergic activity of femoxetine, which has frequently been regarded as a fairly selective 5-HT uptake inhibitor (Bøjholm et al 1979; Reebye et al 1982). In view of the noradrenergic and 5-HT theories of depression (see Introduction), it would be interesting to find whether the weak effect of femoxetine on the noradrenergic system, demonstrated by those and also other authors (Buus Lassen et al 1975; Ghose et al 1977; Petersen 1979), is of importance in its clinical action (Bøjholm et al 1979; Reebye et al 1982).

*Acknowledgements*

We would like to thank the following companies for their generous gifts of substances: Astra Läkemedel AB for H 77/77, norzimelidine and zimelidine, Ciba

Geigy for desipramine and maprotiline, Ferrosan A/S for femoxetine and norfemoxetine, and H. Lundbeck & Co. for citalopram.

## REFERENCES


Åberg, A. (1981) Acta Psychiat. Scand. 63: Suppl. 290: 244–255

Åberg, A., Holmberg, G. (1979) Acta Psychiat. Scand. 59: 45–58

Bruinvels, J. (1971) Br. J. Pharmacol. 42: 281–286

Buus Lassen, J., Squires, R. F., Christensen, J. A., Molander, L. (1975) Psychopharmacologia (Berl.) 42: 21–26

Bøjholm, S., Børup, C., Kvist, J., Petersen, I.-M., le Fevre Honore, P. (1979) Nord. Psykiat. Tidskr. 33: 455–460

Carlsson, A., Corrodi, H., Fuxe, K., Hökfelt, T. (1969) Eur. J. Pharmacol. 5: 367–373

Chang, C. C. (1964) Int. J. Neuropharmacol. 3: 643–649

Coppen, A. (1967) Br. J. Psychiatry 113: 1237–1264

Coppen, A., Rama Rao, V. A., Swade, C., Wood, K. (1979) Psychopharmacology 63: 199–202

Desiles, M., Rips, R. (1981) Br. J. Pharmacol. 74: 81–86

Earley, C. J., Leonard, B. E. (1978) J. Pharmacol. Methods 1: 67–79

Ghose, K., Gupta, R., Coppen, A., Lund, J. (1977) Eur. J. Pharmacol. 42. 31–37

Glowinski, J., Axelrod, J. (1964) Nature 204: 1318–1319

Gottlieb, P., Wandall, T., Fredricson Overø, K. (1980) Acta Psychiatr. Scand. 62: 236–244

Hall, H., Ögren, S.-O. (1981) Eur. J. Pharmacol. 70: 393–407

Hall, H., Sällemark, M., Wedel, I. (1984) Acta Pharmacol. Toxicol. 54: 379–384

Hormazabal, L., Omer, L. M. O., Ismail, S. (1985) Psychopharmacology 86: 205–208

Hyttel, J. (1982) Prog. Neuro-Psychopharmacol. Biol. Psychiat. 6: 277–295

Hyttel, J., Larsen, J.-J. (1985) Acta Pharmacol. Toxicol. 56: Suppl. 1: 146–153

Jones, R. B., Luscombe, D. K. (1977) Postgrad. Med. J. 53: Suppl. 4: 63–76

Lapin, I. P., Oxenkrug, G. F. (1969) Lancet i: 132–136

Maître, L. Moser, P., Baumann, P. A., Waldmeier, P. C. (1980) Acta Psychiatr. Scand. 61: Suppl. 280: 97–110

Maj, J., Stala, L., Górka, Z., Adamus, A. (1982) Psychopharmacology 78: 165–169

Maj, J., Rogóż, Z., Skuza, G., Sowińska, H. (1983) Pol. J. Pharmacol. Pharm. 35: 49–57

Montgomery, S. A. (1980) Neuropharmacology 19: 1185–1190

Montgomery, S. A., McAuley, R., Rani, S. J., Roy, D., Montgomery, D. B. (1981) Acta Psychiat. Scand. 63: Suppl. 290: 314–327

Øfsti, E. (1982) Prog. Neuro-Psychopharmacol. Biol. Psychiat. 6: 327–335

Ögren, S.-O., Ross, S., Hall, H., Archer, T. (1983) in: Burrows, G. D., Norman, T. R., Davies, B. (eds) Antidepressants, Elsevier Science Publishers, Amsterdam; pp 13–34

Pawłowski, L., Kwiatek, H. (1983a) Psychopharmacology 81: 48–53

Pawłowski, L., Kwiatek, H. (1983b) in: Abstracts of the 5th International Catecholamine Symposium, Göteborg, 12–16 June 1983, Progr. Neuro-Psychopharmacol. Biol. Psychiat., Suppl., Pergamon Press, Oxford, pp. 245–246, abstr. 378

Pawłowski, L., Mazela, H. (1984) J. Pharm. Pharmacol. 36: 855–858

Pawłowski, L., Mazela, H. (1986) Psychopharmacology 88: 240–246

Pawłowski, L., Nowak, G., Górka, Z., Mazela, H. (1984) in: Abstracts of the 14th CINP Congress, Florence, 19–23 June 1984, p. 825, abstr. P-602

Pawłowski, L., Nowak, G., Górka, Z., Mazela, H. (1985) Psychopharmacology 86: 156–163

Petersen, E. N. (1979) J. Pharm. Pharmacol. 31: 638–640

Przegaliński, E., Bigajska, K. (1983) Pol. J. Pharmacol. Pharm. 35: 233–240

Przegaliński, E., Siwanowicz, J., Baran, L., Bigajska, K. (1983) Ibid. 35: 309–317

Puech, A. J., Chermat, R., Poncelet, M., Doare, L., Simon, P. (1981) Psychopharmacology 75: 84–91

Reebye, P. N., Yiptong, C., Samsoon, J., Schulsinger, F., Fabricius, J. (1982) Pharmacopsychiatry 15: 164–169

Richelson, E., Pfenning, M. (1984) Eur. J. Pharmacol. 104: 277–286

Slater, I. H., Rathbun, R. C., Kattau, R. (1979) J. Pharm. Pharmacol. 31: 108–110

Träskman, L., Åsberg, M., Bertilsson, L., Cronholm, B., Mellström, B., Neckers, L. M., Sjöqvist, F., Thoren, P., Tybring, G. (1979) Clin. Pharmacol. Ther. 26: 600–610

van Praag, H. M. (1974) Pharmacopsychiatry 7: 281–292

Waldmeier, P. C., Tipton, K. F., Bernasconi, R., Felner, A. E., Baumann, P. A., Maître, L. (1985) Eur. J. Pharmacol. 107: 79–89