Psychopharmacology (1985) 86:156–163 N°1-2

3611

Psychopharmacology

Ro 11-2465 (cyan-imipramine), citalopram and their N-desmethyl metabolites: Effects on the uptake of 5-hydroxytryptamine and noradrenaline in vivo and related pharmacological activities*

L. Pawłowski, G. Nowak, Z. Górka, and H. Mazela**
Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Kraków, Poland

Abstract. Ro 11-2465 (cianopramine, cyan-imipramine) and citalopram (CIT), putative antidepressant drugs, are very potent and selective 5-hydroxytryptamine (5-HT) uptake inhibitors in vitro. This study investigated the effects of these drugs and their desmethyl metabolites, Ro 12-5419 (desmethylcianopramine, cyan-desipramine) and desmethylcitalopram (DCIT), respectively, on the uptake of 5-HT and noradrenaline (NA) in vivo [protection against H 77/77 (4, alpha-dimethyl-metatyramine)-induced displacement of 5-HT and NA] and on related pharmacological activities. All the investigated drugs antagonized H 77/77-induced displacement of 5-HT in the rat brain, though the effects of the metabolites were considerably weaker than those of the parent compounds. The H 77/77-induced displacement of brain NA in rats and mice was antagonized only by Ro 12-5419 and Ro 11-2465. All the drugs potentiated the pressor response to 5-HT in pithed rats; however, Ro 12-5419 and particularly Ro 11-2465 could also block the response when used in higher doses (≧0.1 mg/kg). Only Ro 12-5419 and Ro 11-2465 were able to potentiate the pressor response to NA. Ro 12-5419 also potentiated thyrotropin releasing hormone (TRH) hyperthermia and antagonized reserpine hypothermia in mice; Ro 11-2465 potentiated the TRH hyperthermia only. CIT and DCIT were inactive in both these tests. Of all the four drugs only CIT and Ro 12-5419 considerably stimulated the hind limb flexor reflex in spinal rats. However, whereas the stimulatory effect of CIT was inhibited by the 5-HT antagonists metergoline and cyproheptadine, that of Ro 12-5419 was counteracted by the NA antagonist phenoxybenzamine only. Ro 11-2465, when used in low doses (ca. 1 mg/kg), slightly potentiated the flexor reflex, whereas in higher doses (4–16 mg/kg) it had no effect itself but antagonized the stimulatory action of the 5-HT agonists fenfluramine, quipazine and LSD. The results obtained indicate that Ro 11-2465 and CIT, as well as their desmethyl metabolites, are also potent 5-HT uptake inhibitors in vivo. However, only CIT and DCIT are concurrently devoid of effect on uptake of NA. In contrast, Ro 11-2465 and particularly Ro 12-5419 appear to also inhibit the uptake of NA. Moreover, Ro 11-2465 appears to block central and peripheral 5-HT receptors.

Key words: 5-Hydroxytryptamine uptake inhibition – Noradrenaline uptake inhibition – Ro 11-2465 – Citalopram – Ro 12-5419 – N-desmethyl citalopram – Antidepressant drugs – Rats – Mice

The putative antidepressant drugs Ro 11-2465 (Costa e Silva et al. 1983) and citalopram (CIT) (Gottlieb et al. 1980; Lindegaard Pedersen et al. 1982; Øfsti 1982; Kragh-Sørensen 1983; Mertens 1983) are extremely potent and highly selective 5-HT (5-hydroxytryptamine; serotonin) uptake inhibitors in vitro, more potent and more selective than the classical drug of this type, clomipramine (Hyttel 1977; 1982; Haefely et al. 1978; Burkard 1980; Maître et al. 1980; 1982; Da Prada et al. 1982). Therefore, their positive therapeutic effect (Gottlieb et al. 1980; Lindegaard Pedersen et al. 1982; Øfsti 1982; Costa e Silva et al. 1983; Kragh-Sørensen 1983; Mertens 1983) is attributed to their action on the uptake of 5-HT and is regarded as an important argument for the 5-HT-ergic hypothesis of depression, put forward by Coppen (1967), Lapin and Oxenkrug (1969) and van Praag (1974).

However, the selectivity of Ro 11-2465 seems to be relative and limited only to in vitro conditions; literature data suggest that in vivo Ro 11-2465 may also inhibit the uptake of nor-adrenaline (NA) (Maître et al. 1980, 1982; Pawłowski et al. 1981a; Da Prada et al. 1982). On the other hand, the inhibitory effect of CIT on the uptake of NA in vivo has never been reported, and our own data (Pawłowski et al. 1981b; Pawłowski and Kwiatek 1983a, b), as well as those of the other authors (see above), suggest its selectivity under in vivo conditions.

Like the majority of 5-HT uptake inhibitors, Ro 11-2465 and CIT are tertiary amines and, as such, may be metabolized (demethylation) to respective secondary amines. As a rule, these are less selective, since they inhibit more potently the uptake of NA (Carlsson et al. 1969a, b; Lindbrink et al. 1971; Hyttel 1982; Maj et al. 1982b). For example, desmethylclomipramine, which inhibits the uptake of 5-HT less potently than clomipramine, is simultaneously a very potent NA uptake inhibitor (Hyttel 1982; Maj et al. 1982b), according to Hyttel (1982) even more potent than the classical NA uptake inhibitors desipramine and protriptyline. Due to this fact, the therapeutic action of clomipramine, which is quickly metabolized to desmethyl-

Offprint requests to: L. Pawłowski

* The results were presented at the 14th CINP Congress, Florence, June 19–23, 1984

** *Former name:* Halina Kwiatek

clomipramine which accumulates in the patient's body (Jones and Luscombe 1976; 1977; Träskman et al. 1979), should be regarded as an important argument for the NA-ergic hypothesis of depression (Schildkraut 1965; Schildkraut and Kety 1967) rather than an argument for the 5-HT-ergic one.

Literature data show that desmethylcitalopram (DCIT), which is the main metabolite of CIT in rats, mice, monkeys and in man (Fredricson Overø 1982a, b), is 11 times more potent than the parent drug in inhibiting NA uptake (Hyttel 1977, 1982); concurrently, it is less potent as a 5-HT uptake inhibitor (Hyttel 1977, 1982). Parallel data about the desmethyl metabolite of Ro 11-2465 are not available, as yet.

In view of the above findings, it seemed interesting to us to compare the effects of Ro 11-2465, CIT and their desmethyl metabolites on the uptake of 5-HT and NA in vivo and on related pharmacological activities. In order to measure the ability of these drugs to inhibit the uptake of the monoamines in vivo, we used their antagonism towards H 77/77 (4,alpha-dimethyl-metatyramine)-induced displacement of cerebral 5-HT and NA (Carlsson et al. 1969a, b; Doogan 1980; Maître et al. 1980). The tests generally employed for evaluating antidepressant drugs, such as reserpine hypothermia, TRH hyperthermia, flexor reflex test and blood pressure effects of 5-HT and NA, served as functional models in this study, reflecting the effects of the investigated drugs on 5-HT-ergic and/or NA-ergic processes.

Materials and methods

Animals. Male Wistar rats (180–260 g) and male Albino-Swiss mice (22–36 g) were used throughout. They were kept in colony cages with free access to food (granular standard diet, Bacutil) and tap water until the beginning of the experiment, unless stated otherwise.

Drugs. The drugs administered were: citalopram hydrobromide (CIT) (Lu 10-171-B; H. Lundbeck and Co., København, Denmark), cyproheptadine hydrochloride (Merck, Sharp and Dohme, Rahway, NJ, USA), desipramine hydrochloride (Pertofran; Ciba Geigy, Basel, Switzerland), desmethylcitalopram hydrochloride (DCIT) (Lu 11-109-C; H. Lundbeck and Co., København, Denmark), *d*-fenfluramine hydrochloride (Les Laboratoires Servier, Neuilly-sur-Seine, France), H 77/77 (4,alpha-dimethyl-meta-tyramine-methyl-ester hydrochloride; Astra Läkemedel AB, Södertälje, Sweden), imipramine hydrochloride (Polfa, Starogard Gdański, Poland), LSD (Delysid amp.; Sandoz, Basel, Switzerland), metergoline (Farmitalia, Milano, Italy), noradrenaline (NA) (Levonor amp.; Polfa, Warszawa, Poland), phenoxybenzamine hydrochloride (Smith, Kline and French, Philadelphia, PA, USA), quipazine bimaleate (Miles Laboratories, Inc., Elkhart, IN, USA), reserpine (Rausedyl amp.; Gedeon Richter, Budapest, Hungary), Ro 11-2465 (cyanimipramine hydrochloride, Cianopramine; F. Hoffman-La Roche and Co., Basel, Switzerland), Ro 12-5419 (desmethyl-cyan-imipramine hydrochloride/cyan-desipramine hydrochloride; F. Hoffmann-La Roche and Co., Basel, Switzerland), serotonin creatinine sulfate (5-HT) (Sigma, St. Louis, MO, USA), TRH (thyrotropin releasing hormone, pyroglutamyl-histidyl-prolinamide; Scientific Research Division of the Institute of Chemistry of the University of Gdańsk, Gdańsk, Poland).

Doses refer to the salts given. The drugs were administered intravenously (IV) (into the tail vein) in a volume of 1 ml/kg, intraperitoneally (IP) in a volume of 4 ml/kg (rats) or 10 ml/kg (mice), or subcutaneously (SC) in a volume of 10 ml/kg. Except for metergoline which was suspended in a 1% aqueous solution of Tween 80, cyproheptadine and phenoxybenzamine which were dissolved in warm redistilled water and LSD and reserpine which were dissolved from ampules in redistilled water, all the other drugs were dissolved in 0.9% saline.

H 77/77-induced depletion of brain 5-HT and NA in rats and mice. Animals were injected twice with H 77/77, 12.5 mg/kg IP, the doses being administered 2 h apart. The investigated drugs or saline were administered IP 30 min before each dose of H 77/77, the second dose of the tested drugs being half of the first one. The animals were killed by decapitation 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 N perchloric acid. After centrifugation, 5-HT and NA were separated according to Earley and Leonard (1978); afterwards NA was determined fluorometrically according to the method of Chang (1964), and 5-HT according to the method of Earley and Leonard (1978). 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 of inhibition of monoamine (NA or 5-HT) displacement induced by H 77/77 was calculated using the formula of Bruinvels (1971). The formula takes into account any changes in monoamine (MA) content caused by the test drug when given alone.

$$\% \text{ Inhibition} = \frac{\frac{MA_{drug+H77/77}}{MA_{drug}} - \frac{MA_{H77/77}}{MA_{saline}}}{1 - \frac{MA_{H77/77}}{MA_{saline}}} \times 100.$$

Reserpine hypothermia and TRH hyperthermia in mice. Before the experiment the mice were adapted for 24 h to the conditions (temperature of $20 \pm 1.5°$ C for reserpine-induced hypothermia or $21 \pm 1°$ C for TRH-induced hyperthermia). The rectal body temperature was measured with an Ellab T-3 thermometer at time intervals which are specified elsewhere in the text. The results are expressed as a change in body temperature (Δt) with respect to the initial rectal temperature measured immediately before the injection of investigated drugs. The investigated drugs were administered IP, 20 h after SC injection of reserpine (2.5 mg/kg) or 30 min before IP injection of TRH (40 mg/kg). Each group consisted of 10–12 mice. The statistical significance of results was assessed by Student's *t*-test (two-tailed).

Flexor reflex of the hind limb of the spinal rat. The experiments were performed according to the method described by Maj et al. (1976). The spinal cord was transsected at the level of Th 8–Th 9 under light ether anesthesia. Reflex twitches of the anterior tibial muscle, evoked by electric stimulation (7–20 V, 50–100 ms at 1-min intervals) delivered to the ipsilateral paw from a pair of needle electrodes inserted into the skin of the toes, were recorded. In order to record twitches of the muscle, its distal tendon was attached to an isotonic transducer connected with a pen recorder.

Table 1. The effect of citalopram, Ro 11-2465 (cianopramine, cyanimipramine) and their N-desmethyl metabolites (desmethylcitalopram and Ro 12-5419, respectively) on the displacement of the brain 5-hydroxytryptamine (5-HT) and noradrenaline (NA) by 4,alpha-dimethyl-meta-tyramine (H 77/77) in rats

Treatment	First drug dose (mg/kg/IP)	Drug alone (ng/g ± SEM)	Drug + H 77/77 (ng/g ± SEM)	% Inhibition
Brain 5-HT				
Saline	–	487 ± 16	302 ± 12****	
Citalopram	10	454 ± 18	420 ± 17**	80
Desmethyl-citalopram	10	424 ± 26	337 ± 21	46
Ro 11-2465	10	460 ± 19	417 ± 17**	75
Ro 12-5419	10	456 ± 32	311 ± 12	16
Saline	–	476 ± 15	269 ± 19****	
Citalopram	20	473 ± 29	481 ± 18***	104
Desmethyl-citalopram	20	460 ± 14	374 ± 14***	57
Ro 11-2465	20	486 ± 15	462 ± 8***	89
Ro 12-5419	20	468 ± 11	332 ± 10**	33
Brain NA				
Saline	–	379 ± 17	167 ± 7****	
Citalopram	10	339 ± 12	152 ± 5	−1
Desmethyl-citalopram	10	335 ± 14	158 ± 8	5
Ro 11-2465	10	351 ± 18	177 ± 10	11
Ro 12-5419	10	354 ± 24	185 ± 10	15
Saline	–	369 ± 9	145 ± 6****	
Citalopram	20	346 ± 14	129 ± 6	−3
Desmethyl-citalopram	20	334 ± 13	141 ± 3	5
Ro 11-2465	20	353 ± 14	172 ± 8*	15
Ro 12-5419	20	353 ± 10	201 ± 10***	29

**** $P < 0.001$ when compared with saline value
*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$ when compared with saline + H 77/77 value

Rats were injected with two doses of H 77/77 (12.5 mg/kg IP) 2 h apart. All animals were killed 2 h after the last dose of H 77/77. Test drugs were administered 30 min prior to each dose of H 77/77, the second dose of drug (i.e., 5 or 10 mg/kg IP) being half the first. Each result is the mean ± SEM of seven rats. The statistical significance of the results was assessed by Student's *t*-test

Pressor response to 5-HT and NA in pithed rats. Rats fasted overnight were anesthetized with pentabarbital (30 mg/kg IV). Tracheotomy was then performed and polyethylene cannulae were installed in the carotid artery and tail vein. The CNS was destroyed by the method of Gillespie et al. (1970) and the rat was simultaneously connected to a pump for artificial respiration. Arterial blood pressure was measured in the carotid artery with an EK-4 Farum electromanometer and Statham P23Db transducer. NA and 5-HT were given IV in doses sufficient to induce an increase in blood pressure (Δ blood pressure) by ca. 17 mm Hg (15–20 mm Hg). After obtaining three identical responses, one of the investigated drugs was administered in increasing doses, and 5 min after each dose NA or 5-HT was given twice in the same dose as that established at the beginning of the experiment. In control rats, the responses to the dose of 5-HT or NA chosen at the beginning of the experiment were identical for at least 2 h.

Table 2. The effect of 4,alpha-dimethyl-meta-tyramine (H 77/77) on the brain level of 5-hydroxytryptamine (5-HT) and noradrenaline (NA) in mice. The influence of citalopram, Ro 11-2465 (cianopramine, cyanimipramine) and their N-desmethyl metabolites (desmethylcitalopram and Ro 12-5419, respectively) on the displacement of brain NA induced by H 77/77

Treatment	First drug dose (mg/kg IP)	Drug alone (ng/g ± SEM)	Drug + H 77/77 (ng/g ± SEM)	% Inhibition
Brain 5-HT				
Saline	–	475 ± 38	370 ± 16	
Brain NA				
Saline	–	390 ± 16	175 ± 14****	
Citalopram	10	416 ± 16	163 ± 8	−10
Desmethyl-citalopram	10	362 ± 26	155 ± 9	− 4
Ro 11-2465	10	398 ± 20	204 ± 8	11
Ro 12-5419	10	355 ± 25	211 ± 12	26
Saline	–	383 ± 13	178 ± 16****	
Citalopram	20	400 ± 16	149 ± 10	−17
Desmethyl-citalopram	20	353 ± 6	160 ± 14	− 2
Ro 11-2465	20	358 ± 17	261 ± 18***	49
Ro 12-5419	20	343 ± 10	253 ± 18***	51

**** $P < 0.001$ when compared with saline value
*** $P < 0.001$ when compared with saline + H 77/77 value

Mice were injected with two doses of H 77/77 (12.5 mg/kg IP) 2 h apart. All animals were killed 2 h after the last dose of H 77/77. Test drugs were administered 30 min prior to each dose of H 77/77, the second dose of drug (i.e., 5 or 10 mg/kg IP) being half the first. Each result is the mean ± SEM of six to seven mice. The statistical significance of the results was assessed by Student's *t*-test

Results

H 77/77-induced depletion of the brain 5-HT and NA in rats and mice. H 77/77 markedly decreased the level of brain 5-HT in rats (Table 1) and the level of brain NA in rats and mice (Tables 1, 2). The effect of H 77/77 on the level of brain 5-HT in mice was relatively weak and statistically insignificant (Table 2); this finding is in agreement with the earlier data (Carlsson et al. 1969a, b) that H 77/77 does not displace brain 5-HT in mice.

The potent 5-HT uptake inhibitors CIT and Ro 11-2465 protected rats against the brain 5-HT displacement produced by H 77/77 in a dose-dependent manner (Table 1). DCIT and Ro 12-5419 were also active, though their action was considerably weaker than that of the parent compounds (Table 1). The order of potencies was as follows: CIT ≥ Ro 11-2465 ≫ DCIT ≥ Ro 12-5419 (see Table 1). Of the tested drugs, only Ro 11-2465 and Ro 12-5419 were able to protect animals (rats and mice) against the brain NA displacement caused by H 77/77 (Tables 1, 2); the effect of Ro 12-5419 was slightly stronger. CIT and DCIT did not affect the H 77/77-induced brain NA displacement (Tables 1, 2). None of the investigated drugs had any effect alone on the level of brain 5-HT or NA in rats, or the level of brain NA in mice (Tables 1, 2).

Reserpine hypothermia and TRH hyperthermia in mice. The effects of Ro 11-2465 (10 mg/kg), CIT (10 mg/kg), Ro

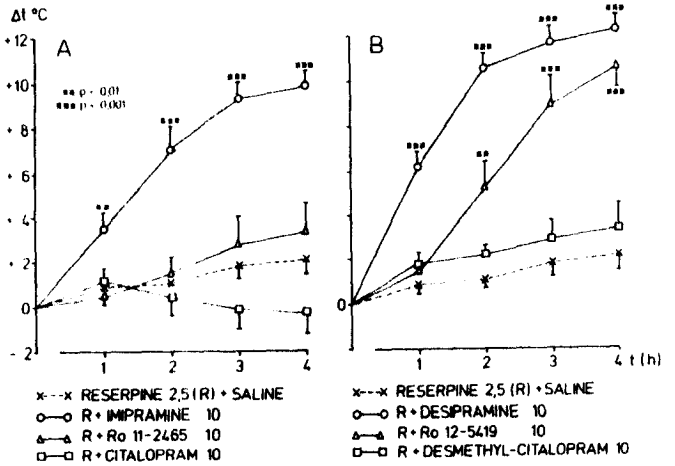

Fig. 1 A, B. The effect of imipramine, Ro 11-2465 and citalopram **A**, and their respective N-desmethyl metabolites desipramine, Ro 12-5419 and desmethylcitalopram **B** upon reserpine (R; 2.5 mg/kg SC)-induced hypothermia in mice. Drugs were given IP (10 mg/kg each) 20 h after reserpine. Each group consisted of 10–12 mice. The results are presented as means ±SEM. The *double* or *triple asterisk* denotes a statistically significant result ($P<0.01$ or 0.001, respectively) in comparison with the control group receiving reserpine alone (Student's t-test, two-tailed). The mean body temperature of control mice at $t=0$ (i.e., 20 h after reserpine injection) was 23.3 ± 0.59° C

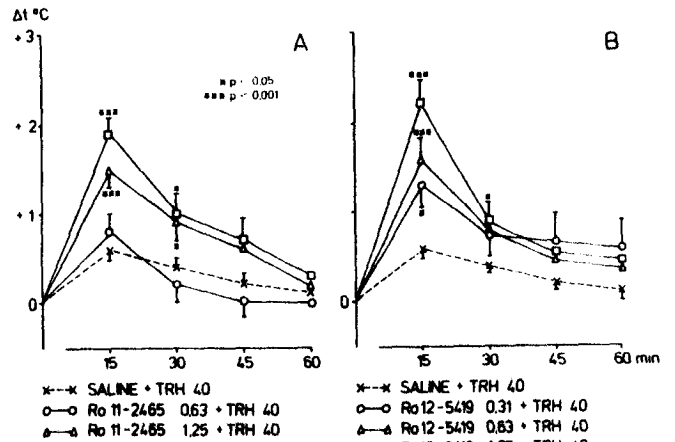

Fig. 2 A, B. The effect of **A** Ro 11-2465 (0.625–2.5 mg/kg IP) and **B** Ro 12-5419 (0.3125–1.25 mg/kg IP) upon TRH (40 mg/kg IP)-induced hyperthermia in mice. The drugs were given 30 min before TRH. Each group consisted of 12 mice. The results are presented as means ±SEM. The *asterisk* or *triple asterisk* denotes a statistically significant result ($P<0.05$ or 0.001, respectively) in comparison with the group receiving TRH alone (Student's t-test, two-tailed)

12-5419 (10 mg/kg) and DCIT (10 mg/kg) on reserpine-induced hypothermia in mice are shown in Fig. 1. For the sake of comparison, Fig. 1 also includes data concerning imipramine (10 mg/kg) and desipramine (10 mg/kg). Of all the tertiary amines shown in this figure, i.e., imipramine, Ro 11-2465 and CIT, only imipramine antagonized reserpine-induced hypothermia (Fig. 1 A); accordingly, of the secondary amines (desmethyl metabolites) only DCIT did not antagonize the hypothermia (Fig. 1 B). CIT (2.5–40 mg/kg and DCIT (2.5–40 mg/kg) were also inactive in the TRH

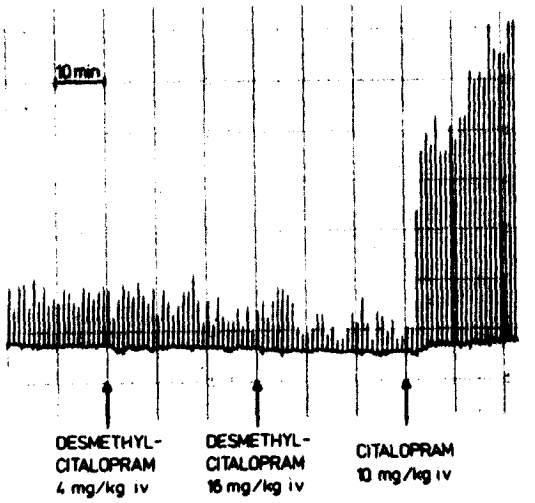

Fig. 3. The effect of desmethylcitalopram (4–16 mg/kg IV) and citalopram (10 mg/kg IV) on the hind limb flexor reflex (reflex contractions of the anterior tibial muscle) in the spinal rat

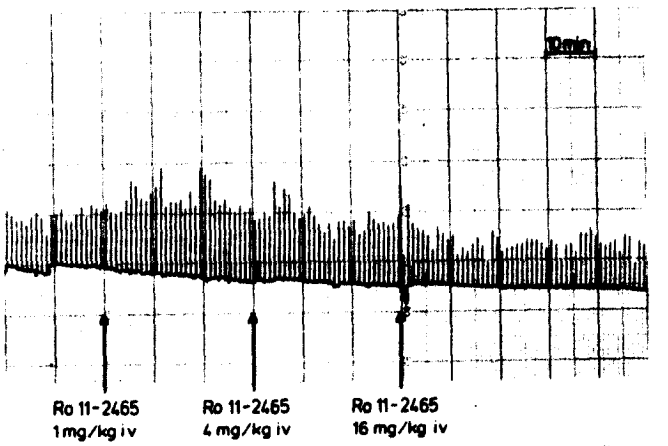

Fig. 4. The effect of Ro 11-2465 (1–16 mg/kg IV) on the flexor reflex in the spinal rat

hyperthermia test, as they did not potentiate TRH (40 mg/kg)-induced hyperthermia in mice (data not shown). In contrast, Ro 11-2465 (0.625–2.5 mg/kg) and Ro 12-5419 (0.3125–1.25 mg/kg) strongly potentiated the hyperthermia induced by TRH, Ro 12-5419 being approximately twice as potent as Ro 11-2465 (Fig. 2).

Flexor reflex of the hind limb of the spinal rat. In agreement with our previous observations (Pawłowski et al. 1981 b), in the experiment reported here CIT (used in doses of 4 and 10 mg/kg) produced a dose-dependent stimulation of the flexor reflex, which was counteracted by standard doses of 5-HT antagonists, i.e., 0.5 mg/kg metergoline or 1 mg/kg cyproheptadine (data not shown). In contrast to CIT, DCIT (1–16 mg/kg) did not stimulate the flexor reflex (Fig. 3); however, even at a dose of 16 mg/kg it did not prevent the stimulatory action of CIT (see Fig. 3) which, as demonstrated earlier (Pawłowski et al. 1981 b), is an indirect 5-HT receptor agonist.

Ro 11-2465, when employed in low doses (ca 1 mg/kg), slightly increased the flexor reflex amplitude (Fig. 4). The same drug used in higher doses (4–16 mg/kg) did not stimulate the flexor reflex (see Fig. 4); however, at those doses

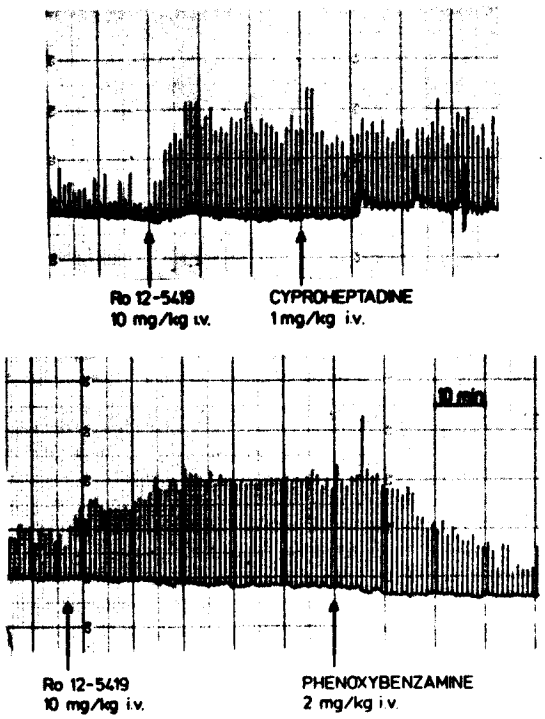

Fig. 5. The effect of cyproheptadine (1 mg/kg IV) or phenoxybenzamine (2 mg/kg IV) on the flexor reflex stimulation induced by Ro 12-5419 (10 mg/kg IV)

it antagonized in a dose-dependent manner the flexor reflex stimulation produced by standard doses of the 5-HT agonists *d*-fenfluramine (1 mg/kg), LSD (0.006 mg/kg) and quipazine (0.1 mg/kg). The latter data are not given, since they are very similar to those reported earlier (Pawłowski et al. 1981a). In contrast to Ro 11-2465, Ro 12-5419 – when used in higher doses (over 4 mg/kg) – considerably stimulated the flexor reflex (Fig. 5). The Ro 12-5419-induced stimulation was unaffected by cyproheptadine (1 mg/kg), but was easily antagonized by a relatively low dose (2 mg/kg) of the alpha-adrenoceptor antagonist phenoxybenzamine (Fig. 5).

Pressor response to 5-HT and NA in pithed rats. CIT (0.01–1 mg/kg) dose-dependently potentiated the pressor response to 5-HT in the pithed rat but had no effect, even at higher doses (0.1–10 mg/kg), on the pressor response to NA (data not shown). DCIT (0.01–10 mg/kg) acted like CIT: it potentiated the pressor response to 5-HT and had practically no effect on the pressor response to NA (Fig. 6). In contrast to CIT and DCIT, Ro 11-2465 (0.01–1 mg/kg) potentiated the pressor response to 5-HT only when employed at the lowest dose; at higher doses (0.1–1 mg/kg) it inhibited the pressor response in a dose-dependent manner. On the other hand, Ro 11-2465 at a dose of 1 mg/kg considerably potentiated the pressor response to NA (data not shown). Ro 12-5419 in doses ranging from 0.01 to 0.1 mg/kg potentiated both the pressor response to 5-HT and the response to NA; however, its effect on the latter response was stronger (Fig. 7). Used in higher doses (1–10 mg/kg), Ro 12-5419 inhibited dose-dependently the pressor response to 5-HT, while still exerting a potentiating

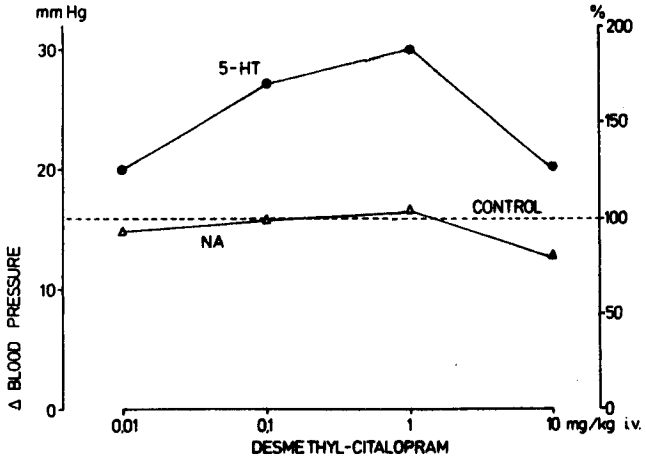

Fig. 6. The effect of desmethylcitalopram (0.01–10 mg/kg IV) on the pressor response to 5-HT and NA in pithed rats. Each symbol represents a mean change in the blood pressure (*Δ* blood pressure) of five to six rats (*left scale*), as well as the percent of the respective controls (*right scale*). *Solid symbols* indicate the significant difference from the control value, obtained at the beginning of the experiment before the drug was injected ($P<0.05$; Student's *t*-test for paired data). The differences between control responses to 5-HT and those to NA were very small (see Methods) and therefore they are not shown in the figure (the *dotted line* represents the two control values – for 5-HT and for NA)

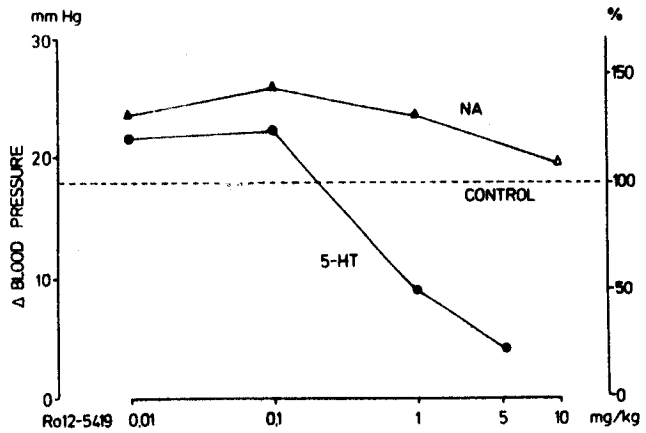

Fig. 7. The effect of Ro 12-5419 (0.01–10 mg/kg) on the pressor response to 5-HT and NA in pithed rats. For other explanations see Fig. 6

effect (though a weaker one) on the pressor response to NA (Fig. 7).

Discussion

The present results confirm and extend our earlier reports (Pawłowski et al. 1981a, b; Pawłowski and Kwiatek 1983a, b) on the effects produced by Ro 11-2465 and CIT and provide some original data about the putative main metabolites of these drugs. As shown in Table 1, Ro 11-2465 and CIT displayed a very potent protective effect against brain 5-HT displacement produced by H 77/77 in rats, an effect which proves their ability to inhibit strongly the uptake of 5-HT in vivo (Doogan 1980). In this respect, their action was similar to that produced by another highly selective and very potent 5-HT uptake inhibitor, fluvoxamine

(Doogan 1980). DCIT and Ro 12-5419 also protected rats against H 77/77-induced 5-HT displacement; however, they were not as potent as the parent drugs. Like fluvoxamine (Doogan 1980), CIT and DCIT did not antagonize the H 77/77-induced NA depletion in the brain. In contrast, Ro 11-2465 and Ro 12-5419 also reduced the NA depletion caused by H 77/77 (Tables 1, 2); this fact, according to several authors (Carlsson et al. 1969a, b; Doogan 1980; Maître et al. 1980), points to their ability to inhibit the neuronal uptake of NA. Therefore, in further experiments we tried to find out the functional evidence for the NA uptake inhibiting action of Ro 11-2465 and Ro 12-5419. CIT and DCIT, which in the biochemical experiment appeared to be 5-HT uptake inhibitors devoid of any effect on the uptake of NA in vivo, served as reference compounds here.

The most striking difference between the action of Ro 11-2465 and Ro 12-5419, and that of CIT and DCIT, was observed in the TRH hyperthermia test and in pressor responses to 5-HT and NA in the pithed rat. In both these tests, Ro 11-2465 and particularly Ro 12-5419 markedly potentiated the effects of NA, i.e., the effect of NA released by TRH (Horst and Spirt 1974) and then involved in the hyperthermia (Pawłowski and Kwiatek 1983a, b), and the effect of NA injected IV (pressor response). Such an action, observed in the above tests, is characteristic of potent inhibitors of the NA uptake (Cavero et al. 1980; Desiles and Rips 1981; Pawłowski and Kwiatek 1983a, b; Pawłowski et al. 1983). As has already been demonstrated here for CIT and DCIT, highly selective 5-HT uptake inhibitors (e.g., fluvoxamine, indalpine, fluoxetine) potentiate neither the TRH-induced hyperthermia (Desiles and Rips 1981; Pawłowski and Kwiatek 1983a, b) nor the pressor response to NA in the pithed rat (Górka, unpublished). On the other hand, 5-HT uptake inhibitors do potentiate the pressor response to 5-HT, unless they concurrently inhibit the vascular 5-HT receptors (Petersen et al. 1977; Pawłowski et al. 1981a, b; Maj et al. 1982a, b).

Ro 12-5419 (but not Ro 11-2465) also behaved as a very potent NA uptake inhibitor in the flexor reflex and reserpine hypothermia tests. It potentiated the flexor reflex via a phenoxybenzamine- (but not cyproheptadine-) sensitive mechanism and antagonized the hypothermia. These effects of Ro 12-5419 therefore resemble those of desipramine, talsupram or oxaprotiline, extremely potent NA uptake inhibitors (for comparison with other drugs of this type, see Hyttel 1982) which not only – like the majority of NA uptake inhibitors – antagonize reserpine-induced hypothermia but also stimulate the flexor reflex by an NA-ergic mechanism (Pawłowski et al. 1983; Pawłowski, unpublished). Less potent NA uptake inhibitors (e.g., maprotiline, see Maitre et al. 1980 and Hyttel 1982) do not stimulate the flexor reflex at all (Pawłowski et al. 1983). Highly selective 5-HT uptake inhibitors do not antagonize reserpine-induced hypothermia (Claassen et al. 1977; Slater et al. 1979; Maj et al. 1983), and when they potentiate the flexor reflex (very potent 5-HT uptake inhibitors only) their effect is easily antagonized by cyproheptadine or metergoline, 5-HT receptor blockers (Pawłowski et al. 1980; 1981a, b; Maj et al. 1982a). Therefore, the negative action of CIT and DCIT in the reserpine hypothermia test and the positive action of CIT (but not DCIT) in the flexor reflex test are in agreement with the biochemical findings (see above) and cited literature data.

In view of the above data, a question arises why Ro 11-2465 does not antagonize the reserpine-induced hypothermia (as a relatively potent NA uptake inhibitor) and why it does not stimulate the flexor reflex (as a very potent 5-HT uptake inhibitor). In the light of our previous (Pawłowski et al. 1981a) and present findings, it seems easy to answer the second question, as we have demonstrated (flexor reflex model, pithed rat preparation) a relatively potent anti-5-HT action of Ro 11-2465; i.e., the latter drug undoubtedly counteracts its own effects due to 5-HT uptake inhibition. The failure of Ro 11-2465 to antagonize the reserpine hypothermia might be explained by antidopamine properties of this drug (Keller et al. 1980), and by the fact that it is such a potent 5-HT uptake inhibitor (Haefely et al. 1978; Burkard 1980; Da Prada et al. 1982; Table 1). As has been recently reported (Maj et al. 1983), 5-HT uptake blockade may counteract the typical action of NA uptake inhibitors in the reserpine hypothermia test; however, the mechanism of this antagonism is unclear, since the action of imipramine (an NA and 5-HT uptake inhibitor) is not potentiated by the blockade of 5-HT receptors (Przegaliński et al. 1983).

In conclusion, the obtained results show that Ro 11-2465 and CIT are very potent 5-HT uptake inhibitors in vivo, and that Ro 11-2465 under in vivo conditions can also inhibit the uptake of NA. These results testify the ability of DCIT and Ro 12-5419 to inhibit the uptake of 5-HT in vivo. They also show that while DCIT is almost as selective as CIT (no effect on the uptake of NA in vivo), Ro 12-5419 should rather be regarded as an NA uptake inhibitor, since its inhibitory action on the uptake of 5-HT is relatively weak, whereas that exerted on the uptake of NA is relatively strong. Thus the pharmacological action of Ro 11-2465 and Ro 12-5419 closely resembles that of clomipramine and desmethylclomipramine, respectively; the latter drugs also potentiate TRH-induced hyperthermia and blood pressure response to NA (and 5-HT) and antagonize the reserpine hypothermia (clomipramine is almost ineffective here) and the biochemical effects induced by NA and 5-HT depletors (Slater et al. 1979; Maître et al. 1980; Maj et al. 1982b; Pawłowski and Kwiatek 1983a, b). In this situation, the positive therapeutic effect of Ro 11-2465 (Costa e Silva et al. 1983) should not be regarded as an important argument for the 5-HT-ergic hypothesis of depression (see Introduction), especially as it may block central 5-HT receptors. On the other hand, CIT, which appears to be a highly selective 5-HT uptake inhibitor in vivo, at least as potent as Ro 11-2465, and whose main metabolite (DCIT) possesses the same pharmacological profile as the parent compound, may be regarded as a useful research tool in depression.

Acknowledgements. We would like to thank the following companies for their generous gift of substances: Astra Läkemedel AB for H 77/77 (4,alpha-dimethyl-meta-tyramine), Ciba-Geigy for desipramine, F. Hoffmann-La Roche and Co. for Ro 11-2465 (cianopramine) and Ro 12-5419 (desmethylcianopramine), H. Lundbeck and Co. for citalopram and desmethylcitalopram, Miles Laboratories, Inc. for quipazine and Polfa for imipramine.

References

Bruinvels J (1971) Evidence for inhibition of the re-uptake of 5-hydroxytryptamine and noradrenaline by tetrahydronaphthylamine. Br J Pharmacol 42:281–286

Burkard WP (1980) Specific binding sites in rat brain for a new and potent inhibitor of 5-hydroxytryptamine uptake: Ro 11-2465. Eur J Pharmacol 61:409–410

Carlsson A, Corrodi H, Fuxe K, Hökfelt T (1969a) Effect of antidepressant drugs on the depletion of intraneuronal brain 5-hydroxytryptamine stores caused by 4-methyl-α-ethyl-meta-tyramine. Eur J Pharmacol 5:357–366

Carlsson A, Corrodi H, Fuxe K, Hökfelt T (1969b) Effects of some antidepressant drugs on the depletion of intraneuronal brain catecholamine stores caused by 4,α-dimethyl-meta-tyramine. Eur J Pharmacol 5:367–373

Cavero I, Gomeni R, Lefèvre-Borg F, Roach AG (1980) Comparison of mianserin with desipramine, maprotiline and phentolamine on cardiac presynaptic and vascular postsynaptic α-adrenoceptors and noradrenaline reuptake in pithed normotensive rats. Br J Pharmacol 68:321–332

Chang CC (1964) A sensitive method for spectrophotofluorometric assay of catecholamines. Int J Neuropharmacol 3:643–649

Claassen V, Davies JE, Hertting G, Placheta P (1977) Fluvoxamine, a specific 5-hydroxytryptamine uptake inhibitor. Br J Pharmacol 60:505–516

Coppen A (1967) The biochemistry of affective disorders. Br J Psychiatry 113:1237–1264

Costa e Silva JA, Omer LMO, Ismail S (1983) Cianopramine, a preliminary efficacy study with a new potent serotonin uptake inhibitor. Abstracts of the 7th World Congress of Psychiatry, Vienna (Austria), 11–16 July 1983, p 489, abstract P223

DaPrada M, Keller HH, Burkard WP, Schaffner R, Bonetti EP, Launay JM, Haefely W (1982) Some neuropharmacological effects of Ro 11-2465 – a novel tricyclic antidepressant with potent inhibitory activity on the uptake of 5-HT. In: Advances in biochemical psychopharmacology vol 31 (Typical and atypical antidepressants: Molecular mechanisms) pp 235–248

Desiles M, Rips R (1981) Use of potentiation of thyrotrophin releasing hormone (TRH)-induced hyperthermia as a test for screening antidepressants which active α-adrenoceptor systems. Br J Pharmacol 74:81–86

Doogan DP (1980) Fluvoxamine as an antidepressant drug. Neuropharmacology 19:1215–1216

Earley CJ, Leonard BE (1978) Isolation and assay of noradrenaline, dopamine, 5-hydroxytryptamine, and several metabolites from brain tissue using disposable Bio-Rad columns packed with Sephadex G-10. J Pharmacol Methods 1:67–79

Fredricson Overø K (1982a) Kinetics of citalpram in test animals; drug exposure in safety studies. Prog Neuro-Psychopharmacol Biol Psychiatr 6:297–309

Fredricson Overø K (1982b) Kinetics of citalopram in man; plasma levels in patients. Prog Neuro-Psychopharmacol Biol Psychiatr 6:311–318

Gillespie JS, Maclaren A, Pollock D (1970) A method of stimulating different segments of the autonomic outflow from the spinal column to various organs in the pithed cat and rat. Br J Pharmacol 40:257–267

Gottlieb P, Wandall T, Fredricson Overø K (1980) Initial, clinical trial of a new, specific 5-HT reuptake inhibitor, citalopram (Lu 10-171). Acta Psychiatr Scand 62:236–244

Haefely W, Schaffner R, Burkard WP, Da Prada M, Keller HH, Pole P, Richards JG (1978) Ro 11-2465, a potent and selective inhibitor of 5-hydroxytryptamine uptake. Abstracts of the 11th CINP Congress. Vienna (Austria), 9–14 July 1978, p 95

Horst WD, Spirt N (1974) A possible mechanism for the antidepressant activity of thyrotropin releasing hormone. Life Sci 15:1073–1082

Hyttel J (1977) Neurochemical characterization of a new potent and selective serotonin uptake inhibitor: Lu 10-171. Psychopharmacology 51:225–233

Hyttel J (1982) Citalopram – pharmacological profile of a specific serotonin uptake inhibitor with antidepressant activity. Prog Neuro-Psychopharmacol Biol Psychiatr 6:277–295

Jones RB and Luscombe DK (1976) Plasma levels of clomipramine and its N-desmethyl metabolite following oral administration of clomipramine in man. Br J Pharmacol 57:430 P

Jones RB and Luscombe DK (1977) Plasma concentrations of clomipramine and its N-desmethyl metabolite in depressive patients following treatment with various dosage regimes of clomipramine. Postgrad Med J 53 (Suppl. 4):63–76

Keller HH, Burkard WP, Da Prada M (1980) Dopamine receptor blockade in rat brain after acute and subchronic treatment with tricyclic antidepressants. Adv Biochem Psychopharmacol vol 24 (Long-term effects of neuroleptics) pp 175–179

Kragh-Sørensen P (1983) A controlled, double-blind multicentre trial comparing clomipramine and a new, highly selective 5-HT uptake inhibitor, citalopram (Lu 10-171). Abstracts of the 7th World Congress of Psychiatry, Vienna (Austria), 11–16 July 1983, p 482, abstract P179

Lapin IP, Oxenkrug GF (1969) Intensification of the central serotoninergic processes as a possible determinant of the thymoleptic effect. Lancet I:132–136

Lindbrink P, Jonsson G, Fuxe K (1971) The effect of imipramine-like drugs and antihistamine drugs on uptake mechanisms in the central noradrenaline and 5-hydroxytryptamine neurons. Neuropharmacology 10:521–536

Lindegaard Pedersen O, Kragh-Sørensen P, Bjerre M, Fredricson Overø K, Gram LF (1982) Citalopram, a selective serotonin reuptake inhibitor: clinical antidepressive and long term effect – A phase II study. Psychopharmacology 77:199–204

Maître L, Baumann PA, Jaekel J, Waldmeier PC (1982) 5-HT uptake inhibitors: psychopharmacological and neurobiochemical criteria of selectivity. Adv Biochem Psychopharm 34 (Serotonin in biological psychiatry):229–246

Maître L, Moser P, Baumann PA, Waldmeier PC (1980) Amine uptake inhibitors: Criteria of selectivity. Acta Psychiatr Scand 61 (Suppl. 280):97–110

Maj J, Palider W, Baran L (1976) The effects of serotonergic and antiserotonergic drugs on the flexor reflex of spinal rat: a proposed model to evaluate the action on the central serotonin receptor. J Neural Transm 38:131–147

Maj J, Rogóz Z, Skuza G (1982a) Fluvoxamine, a new antidepressant drug, fails to show antiserotonin activity. Eur J Pharmacol 81:287–292

Maj J, Rogóz Z, Skuza G, Sowińska H (1983) The effect of selective inhibitors of noradrenaline and serotonin uptake on reserpine- and apomorphine-induced hypothermia in mice. Pol J Pharmacol Pharm 35:49–57

Maj J, Stala L, Górka Z, Adamus A (1982b) Comparison of the pharmacological actions of desmethylclomipramine and clomipramine. Psychopharmacologyy 78:165–169

Mertens C (1983) A controlled double-blind clinical trial with a new highly selective 5-HT-re-uptake inhibitor, citalopram (Lu 10-171) versus mianserin in depressed patients. Abstracts of the 7th World Congress of Psychiatry, Vienna (Austria), 11–16 July 1983, p 441, abstract F924

Pawłowski L, Górka Z, Rogóz Z, Skuza G, Rawlów A, Maj J (1983) Some acute and chronic effects of talsupram, a potent and selective, bicyclic inhibitor of the uptake of noradrenaline. Abstracts of the 5th International Catecholamine Symposium, Göteborg (Sweden), 12–16 June 1983, Prog Neuro-Psychopharmacol Biol Psychiatr (Suppl) p 245, abstract 377

Pawłowski L, Kwiatek H (1983a) The effect of highly selective inhibitors of the uptake of noradrenaline or 5-hydroxytryptamine on TRH-induced hyperthermia in mice. Psychopharmacology 81:48–53

Pawłowski L, Kwiatek H (1983b) TRH-induced hyperthermia in mice: effect of NA and 5-HT uptake inhibitors. Abstracts of the 5th International Catecholamine Symposium, Göteborg (Sweden), 12–16 June 1983, Prog Neuro-Psychopharmacol Biol Psychiatr (Suppl.) p 235, abstract 378

Pawłowski L, Kwiatek H, Górka Z (1981a) Is Ro 11-2465 (cyanimipramine) an antagonist of postsynaptic serotonin receptors? J Neural Transm 52:61–72

Pawłowski L, Kwiatek H, Ruczyńska J (1980) A comparison of

effects of zimelidine with those of other inhibitors of the serotonin (5-HT) uptake. Abstracts of the 12th CINP Congress, Göteborg (Sweden), 22–26 June 1980, Prog Neuro-Psychopharmacol (Suppl.) p 277, abstract 516

Pawlowski L, Ruczyńska J, Górka Z (1981b) Citalopram: A new potent inhibitor of serotonin (5-HT) uptake with central 5-HT-mimetic properties. Psychopharmacology 74:161–165

Petersen EN, Olsson S-O, Squires RF (1977) Effects of 5-HT uptake inhibitors on the pressor response to 5-HT in the pithed rat. The significance of the 5-HT blocking property. Eur J Pharmacol 43:209–215

Przegaliński E, Siwanowicz J, Baran L, Bigajska K (1983) Antagonism by tricyclic antidepressants of reserpine-induced hypothermia in mice. Involvement of postsynaptic α_2-adrenoceptors. Pol J Pharmacol Pharm 35:309–317

Schildkraut JJ (1965) The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am J Psychiatry 122:509–522

Schildkraut JJ, Kety SS (1967) Biogenic amines and emotion. Science 156:21–30

Slater IH, Rathbun RC, Kattau R (1979) Role of 5-hydroxytryptaminergic and adrenergic mechanism in antagonism of reserpine-induced hypothermia in mice. J Pharm Pharmacol 31:108–110

Träskman L, Åsberg M, Bertilsson L, Cronholm B, Mellström B, Neckers LM, Sjöqvist F, Thorén P, Tybring G (1979) Plasma levels of chlorimipramine and its demethyl metabolite during treatment of depression. Clin Pharmacol Ther 26:600–610

van Praag HM (1974) Towards a biochemical typology of depression. Pharmacopsychiatry 7:281–292

Øfsti E (1982) Citalopram – a specific 5-HT-reuptake inhibitor – as an antidepressant drug: A phase II multicentre trial. Prog Neuro-Psychopharmacol Biol Psychiatr 6:327–335

Received July 3, 1984; Final version January 17, 1985

Note added in proof

When this paper had already been accepted for publication, Hyttel and Larsen published a report (Acta Pharmacol. Toxicol. 1985, vol. 56, suppl. 1, pp 146–153) in which they presented data from the in vitro [illegible] CIT and DCIT correspond very well to the in vivo data presented in this paper.