Pharmacopsychiat. 15 (1982) 187–191

Central Action of Amitriptyline N-oxide

J. Maj, J. Vetulani, J. Michaluk, Z. Rogóz, G. Skuza

Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

Summary

The central action of amitriptyline N-oxide (AMINO) has been compared with amitriptyline (AMI) in biochemical and pharmacological studies in rats and mice. It has been found in rats that both drugs prevent 6-OH-dopamine-induced depletion of brain noradrenaline (NA). At the same time AMINO increases and AMI lowers the NA level, both being without effect on 3-methoxy-4-hydroxyphenylglycol concentrations in the brain. AMINO and AMI potentiate the depletion of 5-hydroxytryptamine (5-HT) induced by p-chloroamphetamine in the rat brain and it may be considered as evidence that both drugs do not inhibit 5-HT uptake in vivo. Neither AMINO nor AMI affects the rat brain level of 5-HT but at higher doses they elevate the 5-hydroxy-indoleacetic acid concentrations.

AMINO antagonizes the head twitch reaction induced by 5-hydroxytryptophan in mice and tryptamine convulsions in rats. The hyperthermia induced by fenfluramine (in rats at a high ambient temperature) as well as the stimulation of the hind limb flexor reflex in spinal rats, induced by fenfluramine or LSD, are also inhibited. AMINO antagonizes the 5-HT-induced increase in blood pressure in pithed rats. All the above effects are similar to those induced by AMI, only the active doses of AMINO are higher. The results presented indicate that AMINO, like AMI, inhibits NA uptake and is a 5-HT antagonist.

Zentrale Wirkungsweise von Amitriptyline-N-Oxyd

Die zentrale Wirkung von Amitriptylin-N-Oxid (AMINO) wurde mit der von Amitriptylin (AMI) in biochemischen und pharmakologischen Versuchen an Ratten und Mäusen verglichen. Es wurde an Ratten gefunden, daß beide Substanzen die durch 6-OH-Dopamin bedingte Noradrenalin (NA)-Depletion im Gehirn inhibieren. AMINO erhöht und AMI erniedrigt den Spiegel von Gehirn-NA. Beide haben keinen Einfluß auf die Konzentration von 3-Methoxy-4-hydroxyphenylglykol im Gehirn. AMINO und AMI potenzieren die durch p-Chloramphetamin bedingte 5-Hydroxytryptamin (5-HT)-Depletion im Gehirn der Ratte. Dieser Effekt kann als ein Beweis, daß die beiden Arzneimittel nicht die 5-HT-Aufnahme in vivo hemmen, angesehen werden. AMINO und AMI haben keinen Einfluß auf den Gehirnspiegel von 5-HT, aber erhöhen in höheren Dosen die Konzentration von 5-Hydroxyindolessigsäure. AMINO hemmt die durch 5-Hydroxytryptophan hervorgerufene "head twitch" Reaktion bei Mäusen und die Tryptaminkrämpfe bei Ratten. Es werden auch Hyperthermie nach Fenfluramin (an Ratten bei höherer Umgebungstemperatur) und die durch Fenfluramin oder LSD hervorgerufene Stimulation des Flexorreflexes der hinteren Pfote der spinalen Ratte gehemmt. AMINO wirkt der Blutdruckerhöhung nach 5-HT bei der Ratte mit ausgeschaltetem Zentralnervensystem entgegen. Alle diese obengenannten Effekte sind ähnlich den durch AMI hervorgerufenen, nur die effektiven Dosen von AMINO sind höher. Die vorgestellten Befunde zeigen, daß AMINO, ähnlich wie AMI, die Aufnahme von NA hemmt und ein 5-HT-Antagonist ist.

Amitriptyline (AMINO) has been found to be a clinically effective antidepressant drug (12). Pharmacological studies have shown that AMINO is qualitatively similar to amitriptyline (AMI), its cholinolitic and cardiodepressive action however is weaker (13, 24, 25). Biochemically, the compound has not been extensively investigated. It has only been found that AMINO, as an inhibitor of noradrenaline (NA) and 5-hydroxytryptamine (5.HT) uptake, is considerably weaker than AMI, chiefly under in vitro conditions (1, 10). In this paper, a biochemical investigation of AMINO has been undertaken comparing it to AMI in respect of their action on the central NA and 5-HT systems (brain levels of NA, 5-HT and their principal metabolites, brain NA and 5-HT uptake in vivo). Since we have previously found that AMI is a strong central 5-HT antagonist (16), AMINO has been also studied in this respect.

Methods

The experiments were carried out on male Wistar rats (160–260 g) and male Albino Swiss mice (20–22 g). The animals were kept in colony cages with free access to food and water before experiment were used.

Drugs: Amitriptylinoxide hydrochloride (AMINO; Nattermann et Cie), amitriptyline hydrochloride (AMI; Polfa), p-chloroamphetamine hydrochloride (p-CA; Regis Chemical Co.), 6-hydroxydopamine hydrochloride (6-OHDA; Sigma), L-5-hydroxytryptophan (5.HT; Sigma), fenfluramine hydrochloride (Les Laboratoires Servier), lysergic acid diethylamide (LSD; Spofa), noradrenaline hydrotartrate (NA; Polfa), tryptamine hydrochloride (Polfa) were used. AMINO and 5-HTP were suspended in 1% aqueous solution of Tween 80. 6-OHDA was dissolved in saline containing 0.1% of ascorbic acid. Other drugs were administered as saline solutions.

Statistics: In biochemical studies the significance of effects was evaluated by analysis of variance, followed, if appropriate, by specific comparisons (Newman-Keuls or Dunnett test). In pharmacological studies Student's t-test was used and the ED_{50} values with confidence limits were calculated according to *Litchfield* and *Wilcoxon* (14).

Brain levels of NA, 3-methoxy-4-hydroxyphenylglycol (MHPG), 5-HT and 5-hydroxyindole acetic acid (5-HIAA) in rats

AMINO (20 and 50 mg/kg i.p.) and AMI (5 and 20 mg/kg i.p.) were administered 1 h before killing (by decapitation) the animals. Whole brains were excised and stored under solid CO_2 until used in biochemical assay. NA, 5-HT and 5-HIAA were assayed spectrofluorometrically after isolation from brain homogenates and separation on a Sephadex G-10 column (4). MHPG was assayed by a gaschromatographic method of *Braestrup* (2), using vanilmandelic acid as the internal standard.

Brain NA and 5-HT uptake in vivo in rats

Antagonism of AMINO and AMI towards 6-OHDA-induced depletion of brain NA or towards p-CA-induced decrease in brain 5-HT was taken as a measure of NA or 5-HT uptake inhibition in vivo respectively. AMINO (20 and 50 mg/kg i.p.) and AMI (5 and 20

0720–4280/82 1132–0187 $ 02.00

Table 1 The effect of AMINO and AMI on cerebral levels of NA and MHPG. MHPG was assayed in two experimental series (a and b) that yielded slightly different control values; percentages were calculated from respective controls. The concentrations (means ± S.E.M.) are given in ng/g tissue. Number of animals given in parentheses. Individual comparisons between means were carried out by the Newman-Keuls test

Treatment, dose (mg/kg	NA	%	MHPG	%	Experiment
Saline	393 ± 16 (7)	100	145 ± 8 (5)	100	a
			135 ± 7 (5)	100	b
AMINO, 20	420 ± 26 (7)	107	134 ± 9 (5)	100	b
AMINO, 50	520 ± 18 (7)	132**	133 ± 4 (5)	98	b
AMI, 5	317 ± 32 (7)	81*	142 ± 7 (5)	97	a
AMI, 20	413 ± 32 (7)	105	114 ± 7 (5)	79	a
	F = 9.85 df 4/30		F = 2.29 df 5/24		
	$p < 0.001$		N.S.		

* $p < 0.05$, ** $p < 0.01$

mg/kg i.p.) were injected 1 h before administration of an amine depleting agent. 6-OHDA was injected in a dose of 250 µg, in a volume of 20 µl, into the lateral brain ventricle of non-anesthetized rats, as described earlier (23). p-CA was given in a dose of 10 mg/kg i.p. Rats were killed (as above) 6 days after 6-OHDA or 4 h after p-CA. Whole brain NA and 5-HT levels were assayed as above.

5-HTP-induced head twitch reaction in mice

The influence of AMINO (i.p., 1 h before 5-HTP) on 5-HTP (280 mg/kg i.p.)-induced head zwitch reaction was studied in mice according to *Corne* et al. (3). The dose of AMINO required to decrease by 50% the mean frequency of head twitch reaction (ED_{50}) was estimated. Six mice were used at each of 5 dose levels of AMINO.

Tryptamine-induced clonic convulsions of forepaws in rats

Clonic convulsions of forepaws in rats were evoked by tryptamine (40 mg/kg i.v.; the dose refers to the base) according to *Tedeschi* et al. (22). The effect was assayed for 5 min after tryptamine using a 3-point scale (20). AMINO was administered i.p. 1 h before tryptamine and the dose required to decrease by 50% the mean rating score (ED_{50}) was estimated. Eight rats were used at each of 4 dose levels of AMINO.

Fenfluramine-induced hyperthermia in rats kept at a high ambient temperature

The experiments were carried out according to *Sulpizio* et al. (21) at an ambient temperature of 28 ± 1°C. Esophageal body temperature was measured with an electric Ellab thermometer for 3 h (at 30 min intervals) after administration of fenfluramine (20 mg/kg i.p.). AMINO and AMI (both in doses of 1, 5 and 20 mg/kg i.p.) were injected 1 h before fenfluramine. Each group consisted of 6 rats.

Fenfluramine- or LSD-induced stimulation of the hind limb flexor reflex in spinal rats

Experiments were carried out according to *Maj* et al. (17). The rat hind paw was stimulated through platinum electrodes inserted into the skin of the foot using electrical impulses (6–12 V, 60–120 msec, at 1 min intervals). The concentrations of the musculus tibialis anterior were recorded. The stimulation of the flexor reflex was induced by fenfluramine (1 mg/kg) or LSD (3 µg/kg). AMINO (1, 2.5 and 5 mg/kg) and AMI (0.8 and 1 mg/kg) were administered 30 min before fenfluramine or LSD. All the drugs were injected i.v.

5-HT- or NA-induced pressor response in pithed rats

In pithed rats arterial blood pressure from the right carotid artery was recorded. Pressor responses were induced by 5-HT or NA given in doses (0.005–0.015 mg/kg or 0.0001–0.001 mg/kg respectively) producing the increase in blood pressure by about 15 mm Hg. AMINO and AMI (both in doses 0.01–10 mg/kg) were administered 5–10 min before 5-HT or NA. All the drugs were injected i.v.

Results

Brain levels of NA, MHPG, 5-HT and 5-HIAA in rats

AMINO and AMI did not significantly affect the MHPG level, while they produced changes in NA level: the high dose of AMINO (50 mg/kg) elevated the cerebral concentration of NA, while the low dose of AMI (5 mg/kg) depressed it. Although significantly different from control values, the changes were moderate (20–30%) (Table 1).

AMINO and AMI did not significantly affect the cerebral levels of 5-HT; the brain concentration of 5-HIAA was significantly elevated by a high dose only (50 mg/kg) of AMINO (Table 2).

Table 2 The effect of AMINO and AMI on cerebral levels of 5-HT and 5-HIAA. The concentrations (means ± S.E.M.) are given in ng/g tissue. Number of animals given in parentheses. Individual comparisons between means were carried out by the Newman-Keuls test

Treatment, dose (mg/kg)	5-HT	%	5-HIAA	%
Saline	340 ± 29 (8)	100	196 ± 23 (8)	100
AMINO, 20	316 ± 22 (8)	93	251 ± 29 (8)	128
AMINO, 50	393 ± 16 (8)	116	397 ± 30 (8)	203*
AMI, 5	286 ± 36 (8)	84	189 ± 35 (6)	97
AMI, 20	365 ± 36 (8)	107	275 ± 35 (8)	141
	F = 2.06 df 4/35		F = 7.61 df 4/33	
	N.S.		$p < 0.001$	

* $p < 0.01$

Brain NA and 5-HT uptake in vivo in rats

AMINO and AMI offered partial protection against the depleting action of 6-OHDA; the effects of higher dose (50 and 20 mg/kg, respectively) reached the level of statistical significance (Table 3).

AMINO (50 mg/kg) and AMI (5 and 20 mg/kg) significantly potentiated p-CA-induced depletion of brain 5-HT) (Table 4).

5-HTP-induced head twitch reaction in mice

AMINO antagonized 5-HTP-induced head twitch reaction in mice. Its ED_{50} value is 24.1 (7.58–76.34 mg/kg i.p.). For comparison ED_{50} of AMI is 1.3 (0.76–2.21) mg/kg i.p. (16).

Table 3 The effect of AMINO and AMI on 6-OHDA-induced depletion of brain NA. The concentrations (means ± S.E.M.) are given in ng/g tissue. Number of animals given in parentheses. Individual comparisons between means were carried out with the one-sided Dunnett test.

Pretreatment, dose (mg/kg)	Treatment	NA	%
Saline	Solvent	403 ± 28 (7)	100
Saline	6-OHDA	93 ± 16 (10)	23[a]
AMINO, 20	6-OHDA	136 ± 19 (10)	34[a]
AMINO, 50	6-OHDA	170 ± 25 (9)	42[a, b]
AMI, 5	6-OHDA	116 ± 7 (9)	29[a]
AMI, 20	6-OHDA	152 ± 17 (9)	38[a, b]
		F = 29.2 df 5/48	
		p < 0.001	

a—p < 0.01 (vs. saline + solvent group), b—p < 0.05 (vs. saline + 6-OHDA group)

Table 4 The effect of AMINO and AMI on p-CA-induced depletion of brain 5-HT. The concentrations (means ± S.E.M.) are given in ng/g tissue. Number of animals in parentheses. Individual comparisons between means were carried out with the one-sided Dunnett test

Pretreatment, dose (mg/kg)	Treatment	5-HT	%
Saline	Saline	323 ± 16 (8)	100
Saline	p-CA	200 ± 21 (7)	62[a]
AMINO, 20	p-CA	166 ± 26 (8)	51[a]
AMINO, 50	p-CA	141 ± 14 (8)	44[a, b]
AMI, 5	p-CA	141 ± 18 (8)	44[a, b]
AMI, 20	p-CA	127 ± 18 (8)	39[a, b]
		F = 16.30 df 5/41	
		p < 0.001	

a—p < 0.01 (vs. saline + saline group), b—p < 0.05 (vs. saline + p-CA group)

Tryptamine-induced clonic convulsions of forepaws in rats

AMINO inhibited tryptamine-induced clonic convulsions of forepaws in rats and its ED_{50} value is 20.1 (7.22–55.86) mg/kg i.p. As reported previously (16) ED_{50} of AMI was 4.7 (2.65–8.13) mg/kg i.p.

Fenfluramine-induced hyperthermia in rats kept at a high ambient temperature (Table 5)

AMINO 1 and 5 mg/kg slightly but significantly antagonized fenfluramine-induced hyperthermia and this effect was shown only at 30 min after fenfluramine administration. The antagonistic effect of the highest dose of AMINO (25 mg/kg) lasted up to 90 min after fenfluramine.

AMI 1 mg/kg was ineffective, but given in doses of 5 and 25 mg/kg antagonized fenfluramine-induced hyperthermia. The effect was dose dependent and lasted for 3 hrs after administration of fenfluramine.

Fenfluramine- or LSD-induced stimulation of the hind limb flexor reflex in spinal rats

AMINO, 1 and 2.5 mg/kg was ineffective, however, if given in a dose of 5 mg/kg, it prevented completely the stimulation of the flexor reflex induced by fenfluramine or LSD (Figs. 1 and 2).

AMI produced a similar effect when given in doses 0.8–1 mg/kg (Fig. 3).

5-HT- or NA-induced pressor response in pithed rats

AMINO, 0.01–1 mg/kg did not affect 5-HT-induced pressor response. The drug given in doses of 3–10 mg/kg re-

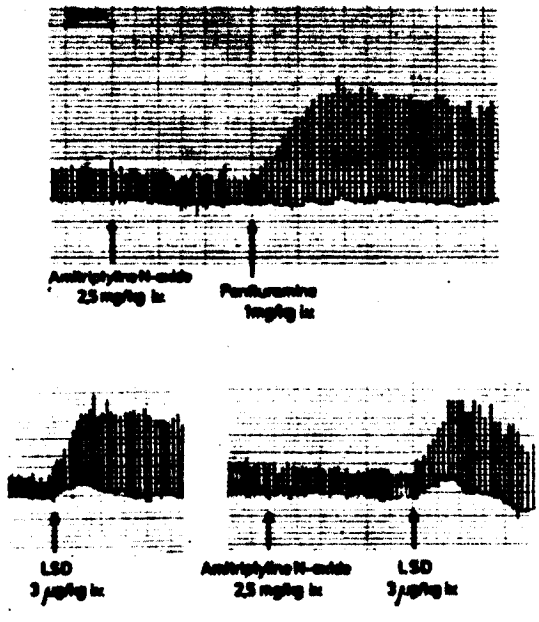

Fig. 1 Effect of amitriptyline N-oxide (2.5 mg/kg i.v.) on fenfluramine- or LSD-induced stimulation of hind limb flexor reflex of the spinal rat.

Table 5 Effect of AMINO and AMI on fenfluramine-induced hyperthermia in rats at ambient temperature of 28°C

Drugs and doses mg/kg	Initial temp. °C	Δ t (°C) 30 min	60 min	90 min	120 min	150 min	180 min
Fenfluramine (FF) 20	37.0 ± 0.1	+2.7 ± 0.3	+2.9 ± 0.2	+2.9 ± 0.2	+2.4 ± 0.2	+2.3 ± 0.2	+2.0 ± 0.2
AMINO 1 + FF 20	37.1 ± 0.1	+1.7 ± 0.2[b]	+2.4 ± 0.2	+2.2 ± 0.2	+1.9 ± 0.2	+2.0 ± 0.2	+1.6 ± 0.2
AMINO 5 + FF 20	36.9 ± 0.2	+1.8 ± 0.1[b]	+2.5 ± 0.2	+2.2 ± 0.2	+1.9 ± 0.2	+1.8 ± 0.2	+1.6 ± 0.2
AMINO 25 + FF 20	37.0 ± 0.1	+0.9 ± 0.4[c]	+1.5 ± 0.3[c]	+2.1 ± 0.2[a]	+2.0 ± 0.2	+1.9 ± 0.2	+1.6 ± 0.2
FF 20	37.2 ± 0.2	+1.4 ± 0.3	+2.1 ± 0.2	+2.1 ± 0.2	+2.2 ± 0.1	+2.4 ± 0.1	+2.6 ± 0.2
AMI 1 + FF 20	37.3 ± 0.1	+1.1 ± 0.4	+1.6 ± 0.4	+1.9 ± 0.3	+1.7 ± 0.3	+1.5 ± 0.2	+1.4 ± 0.3
AMI 5 + FF 20	37.1 ± 0.1	+0.8 ± 0.1	+1.3 ± 0.2[a]	+1.7 ± 0.3[a]	+1.6 ± 0.3[a]	+1.5 ± 0.3[b]	+1.3 ± 0.3[c]
AMI 25 + FF 20	37.4 ± 0.2	−0.2± 0.2[d]	+0.3 ± 0.2[d]	+0.7 ± 0.2[d]	+0.7 ± 0.2[d]	+0.7 ± 0.2[d]	+0.7 ± 0.2[d]

[a]p < 0.05; [b]p < 0.02; [c]p < 0.01; [d]p < 0.001

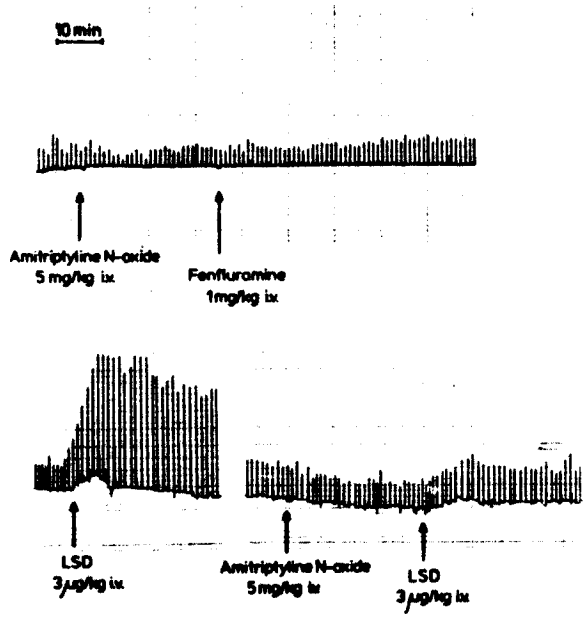

Fig. 2 Effect of amitriptyline N-oxide (5 mg/kg i.v.) on fenfluramine- or LSD-induced stimulation of hind limb flexor reflex of the spinal rat

duced it in a dose-dependent manner with ED_{50} value about about 6 mg/kg. AMI inhibited pressor response to 5-HT in doses of 0.01–3 mg/kg with ED_{50} value about 0.03 mg/kg.

AMINO at doses of 0.01–1 mg/kg did not modify the pressor response to NA. Higher doses (3–10 mg/kg) slightly potentiated the response. Maximum response (30%) was observed after the dose of 10 mg/kg. AMI (0.01–10 mg/kg) enhanced the pressor response to NA, with ED_{50} value about 1 mg/kg.

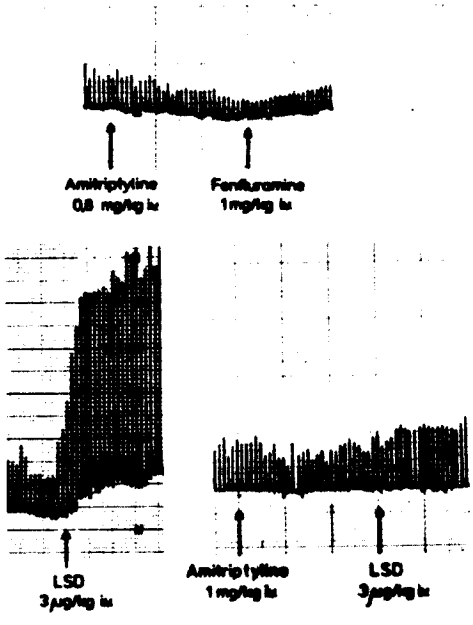

Fig. 3 Effect of amitriptyline on fenfluramine- or LSD-induced stimulation of hind limb flexor reflex of the spinal rat

Discussion

Our pharmacological and biochemical studies indicate that AMINO is an antagonist of 5-HT and an inhibitor of NA uptake. The central anti-5-HT action of AMINO is shown by the inhibition of head twitch reaction induced by 5-HTP and tryptamine convulsions, by the antagonism to LSD in the hind limb flexor reflex preparation and also by the antagonism towards fenfluramine found in the flexor reflex preparation and in the hyperthermia test. It is noteworthy, however, that the effects of fenfluramine (a 5-HT releaser) are antagonized not only by 5-HT antagonists but also by 5-HT uptake inhibitors as demonstrated in several pharmacological (6, 21) and biochemical (8, 18) studies. On the other hand the inhibition of 5-HT uptake by AMINO in vivo is not very likely if the results of the biochemical experiments (see below) are taken into consideration.

Another main property of AMINO is NA uptake inhibition. This is indicated first of all by the protection against the depletion of brain NA, induced by 6-OHDA. Accordingly, such a protection was shown for several NA uptake inhibitors (5, 26).

Although AMINO has been reported to inhibit 5-HT uptake in vitro (1, 10), we were not able to confirm it in our in vivo experiments. Thus – in contrast to known 5-HT uptake inhibitors (18, 27) – AMINO not only did not protect against p-CA-induced depletion of brain 5-HT but – on the contrary – potentiated it. The latter effect is difficult to explain. However, one could expect it to be a result of increased 5-HT turnover due to – via a feedback mechanism – 5-HT antagonistic activity of AMINO. In fact, such an effect has been demonstrated for some 5-HT antagonists (9, 11) and the rise in brain 5-HIAA level may be regarded as an evidence for the increase in 5-HT turnover after AMINO.

It should be added that AMI, like AMINO, potentiates also the p-CA-induced depletion of brain 5-HT. This effect indicates that AMI does not inhibit the 5-HT uptake in vivo, which is in accordance with literature data (7, 26).

Thus our results taken as a whole show that under in vivo conditions AMINO does not inhibit 5-HT uptake. Even if such an effect does exist it is weak and from the functional point of view has no meaning. 5-HT antagonistic activity of AMINO seems to be considerably stronger and this leads to inhibition of serotoninergic transmission which can not be surmounted by weak serotoninergic activation resulting from the uptake inhibition.

All the above considerations refer to the central action of AMINO. Our experiments with pithed rats demonstrate that the peripheral effects of the drug are similar (NA uptake inhibition and 5-HT antagonistic activity). The potentiation of the pressor response to NA and epinephrine in anaesthetized cats and the antagonism towards 5-HT in the isolated small intestine of guinea-pigs has also been reported for AMINO by *Leuschner* et al. (13).

The present and earlier (16) studies indicate that the pharmacological profile and biochemical effects of AMINO are similar to those of AMI. The observed differences are of

quantitative nature. In the central nervous system AMINO acts in doses a few times higher than AMI and the differences between the effective doses of both drugs depend on the test. For most of the pharmacological tests used to evaluate the anti-5-HT activity in the rat, the effective doses of AMINO are about 5 times higher than those of AMI. In the 6-OHDA test (the NA uptake inhibition in vivo) these differences are smaller and amount to an index value of 2–2.5. The greatest differences exist for peripheral action (pithed rat). AMINO acts 10 times more weakly as NA uptake inhibitor and 200 times more weakly as 5–HT antagonist. This indicates that the peripheral side effects (e.g. cardiodepressive effects) after AMINO may be weaker than those after AMI.

The similarity in the pharmacological profiles of AMINO and AMI may be the result of two facts. Firstly, both drugs are very similar in their chemical structure. Secondly, AMINO is, at least in rats, metabolished to AMI (19), it is therefore possible that the effects observed for AMINO may results from the action of the metabolite, AMI. It is worth to add that after prolonged treatment with AMINO a high and very stable AMI concentration in the brain (more stable than after treatment with AMI) is observed (19); this may be meaningful from the clinical point of view.

In conclusion, as far as the pharmacological profile of AMINO is concerned, two basic properties are conspicuous, i.e. NA uptake inhibition and 5-HT antagonism. Both properties may be important for the antidepressant activity of AMINO, as both lead to activation of the NA system. A considerable amount of data indicate that an interaction between the NA and 5-HT systems exists in the brain. Thus, the blockade of the 5-HT system changes the normally existing equilibrium in favour of the NA system (see: 15).

Acknowledgements

The authors wish to thank Nattermann et Cie (amitriptylinoxide), Polfa (amitriptyline), Servier (fenfluramine) for generously providing the drugs.

References

1 *Bertilsson, L., B. Mellström, F. Sjöqvist:* Pronounced inhibition of noradrenaline uptake by 10-hydroxy-metabolites of nortriptyline. Life Sci. 25 (1979) 1285–1292
2 *Braestrup, C.:* Identification of free and conjugated 5-methoxy-4-hydroxyphenylglycol (MOPEG) in rat brain by gaschromatography and mass fragmentography. Anal. Biochem. 55 (1973) 420–431
3 *Corne, S.J., R.W. Pickering, B.T. Warner:* Method for assessing the effect of drugs on the central action of 5-hydroxytryptamine. Br. J. Pharmacol. 20 (1963) 106–120
4 *Earley, C.J., B.E. Leonard:* 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. Meth. 1 (1978) 67–79
5 *Evetts, K.D., L.L. Iversen:* Effects of protriptyline on the depletion of catecholamines induced by 6-hydroxydopamine in the brain of the rat. J. Pharm. Pharmacol. 22 (1970) 540–542
6 *Frey, H.H.:* Hyperthermia induced by amphetamine, p-chloroamphetamine and fenfluramine in the rat. Pharmacology 13 (1975) 163–176
7 *Fuller, R.W., K.W. Perry, B.B. Molloy:* Effect of 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine on the depletion of brain serotonin by 4-chloroamphetamine. J. Pharmacol. Exp. Ther. 193 (1975) 796–803
8 *Ghezzi, D., R. Samanin, S. Bernasconi, G. Tognoni, M. Gerna, S. Garattini:* Effect of thymoleptics on fenfluramine-induced depletion of brain serotonin in rats. Eur. J. Pharmacol. 24 (1973) 205–210
9 *Gołembiowska-Nikitin, K., G. Wiszniowska, J. Marchaj:* The effect of serotonin receptor blocking agents – cyproheptadine and danitracen – on serotonin turnover in the rat brain. Pol. J. Pharmacol. Pharm. 29 (1977) 485–495
10 *Hyttel, J., A.V. Christensen, B. Fjalland:* Neuropharmacological properties of amitriptyline, nortriptyline and their metabolites. Acta Pharmacol. Toxicol. 47 (1980) 53–57
11 *Jacoby, H.J., J. Shabshelowitz, J.D. Fernstrom, R.J. Wurtman:* The mechanism by which methiothepin, a putative serotonin receptor antagonist, increases brain 5-hydroxyindole levels. J. Pharmacol. exp. Ther. 195 (1975) 257–264
12 *Kanig, K., K. Tuluweit:* Synopsis of results of clinical trials with amitriptylinoxide. Arzneimittel-Forsch. 28 (1978) 1920–1923
13 *Leuschner, F., W. Neumann, I.S. Doppelfeld, A. Erdös, R. Fontaine, H.A. Gabbar, E. Graf:* General pharmacology of amitriptylinoxide. Arzneimittel.Forsch. 28 (1978) 1883–1893
14 *Litchfield, J.T., F. Wilcoxon:* A simplified method of evaluating dose – effect experiments. J. Pharmacol. Exp. Ther. 96 (1949) 99–113
15 *Maj, J.:* Serotonin Mechanism of Antidepressant Drugs. Pharmacopsychiatria 14 (1981) 35–39
16 *Maj, J., A. Lewandowska, A. Rawłów:* Central antiserotonin action of amitriptyline. Pharmacopsychiat. 11 (1979) 281–285
17 *Maj, J., W. Palider, L. Baran:* The effects of serotonergic and antiserotonergic drugs on the flexor reflex on spinal rat: a proposed model to evaluate the action on the central serotonin receptor. J. Neural. Transm. 38 (1976) 131–147
18 *Meek, J.L., K. Fuxe, A. Carlsson:* Blockade of p-chloromethamphetamine induced 5-hydroxytryptamine depletion by chlorimipramine, chlorpheniramine and meperidine. Biochem. Pharmacol. 20 (1971) 707–709
19 *Melzacka, M., L. Danek:* Pharmacokinetics of amitriptyline N-oxide in rats after acute and chronic treatment, C.I.N.P. 13th Congress 1982, Abstracts pp
20 *Niemegeers, C.J.E., F.M. Lenaerts, K.S.K. Artois, P.A.J. Janssen:* Interaction of drugs with apomorphine, tryptamine and norepinephrine. A new insvivo approach: the ATN-test in rats. Arch. Int. Pharmacodyn. Thér. 227 (1977) 238–253
21 *Sulpizio, A., P.J. Fowler, E. Macko:* Antagonism of fenfluramine-induced hyperthermia: ameasure of central serotonin inhibition. Life Sci. 22 (1978) 1439–1446
22 *Tedeschi, D.H., E.R. Tedeschi, E.J. Fellows:* The effect of tryptamine on the central nervous system including a pharmacological procedure for the evaluation of iproniazid-like drugs. J. Pharmacol. Ex. Ther. 126 (1959) 223–232
23 *Vetulani, J., K. Reichenberg, G. Wiszniowska:* Asymmetric behavioral and biochemical effects of unilateral injections of 6-hydroxydopamine into the lateral brain ventricle of the rat. Eur. J. Pharmacol. 19 (1972) 231–238
24 *Wenzl, H.:* Special experiments on central nervous effects of amitriptylinoxide considering pharmacokinetic aspects. Arzneimittel-Forsch. 28 (1978) 1879–1883
25 *Wenzl, H., E. Graf, A. Sieck:* Central nervous effects of a new tricyclic antidepressant (amitriptylinoxide) on laboratory animals. Arzneimittel-Forsch. 28 (1978) 1874–1878
26 *Wong, D.T., F.P. Bymaster:* The comparison of fluoxetine and nisoxetine with tricyclic antidepressants in blocking theneurotoxicity of p-chloroamphetamine and 6-hydroxydopamine in the rat brain. Res. Commun. Chem. Pathol. Pharmacol. 15 (1976) 221–231
27 *Wong, D.T., J.-S. Horng, R.W. Fuller:* Kinetics of serotonin accumulation into synaptosomes of rat brian: effect of amphetamine and chloroamphetamine. Biochem. Pharmacol. 22 (1973) 311–322

J. Maj, M.D., Institute of Phrarmacology, Polish Academy of Sciences, 31-343 Kraków, Poland