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OXAPROTILINE, A NORADRENALINE UPTAKE INHIBITOR WITH AN ACTIVE AND AN INACTIVE ENANTIOMER

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Abstract—The new antidepressant drug, oxaprotiline, and its two enantiomers were investigated with respect to their effects on noradrenaline (NA) and serotonin (5-HT) uptake *in vitro* and *in vivo* after acute and repeated treatment. Moreover, the alpha-adrenolytic effects *in vitro* were also studied. Oxaprotiline proved to be a highly potent and selective inhibitor of NA uptake in rat synaptosomal preparations *in vitro* and in the rat heart and brain *in vivo*. The NA uptake-inhibiting properties were found to be confined entirely to the (+)- or S-enantiomer; (−)- or R-oxaprotiline, the absolute configuration of which corresponds to that of the naturally occurring (−)-NA, was about 1000 times less potent than the (+)-form *in vitro*, and was inactive *in vivo* at doses exceeding the ED_{50} of the latter 100-fold. The selectivity of oxaprotiline with respect to NA uptake inhibition was retained after 10 daily administrations. No sign of cumulation or attenuation of the effect was evident. No uptake-inhibiting effect of (−)-oxaprotiline appeared after 10 daily administrations of high doses, indicating that no racemization occurred in the organism. The α_1-adrenoceptor antagonistic effect of oxaprotiline, as determined by the ability to displace [^{3}H]prazosin, was in the range of that of imipramine, the (−)-enantiomer being somewhat more potent than the (+)-form. In contrast to imipramine, oxaprotiline was devoid of α_2-adrenoceptor antagonistic effects, as judged by the ability to affect the impulse-related release of [^{3}H]NA from rat cortical slices. Since oxaprotiline proved to be an effective antidepressant, clinical testing of its two enantiomers might be helpful with respect to the validation of the catecholamine hypothesis of depression. Moreover, in animal studies, they might help to determine which effects of antidepressants are related to NA uptake inhibition and which are not.

The catecholamine hypothesis of depression [1–3] was based in essence on the pharmacological actions of the antidepressant agents, which inhibit the degradation of catecholamines (MAO inhibitors) or their elimination from the synaptic cleft (uptake inhibitors). There has been and still is considerable criticism of the monoamine hypotheses of affective disorders in general and of the noradrenaline (NA) hypothesis in particular [4–7], the major point of attack being the latency of the onset of the antidepressant effect of the drugs, in contrast to their immediate pharmacological actions.

On the other hand, progress has been made in recent years in the understanding of changes in transmitter synthesis or release and receptor function, occurring secondary to alterations in synaptic transmitter concentrations induced by antidepressant agents. Adaptational phenomena have been described at the level of NA turnover [8–10] as well as at its pre- and postsynaptic receptors [11–14], which could well explain why the antidepressant effect does not show a time-course comparable to that of the acute pharmacological effects (for a discussion see [15]).

In consequence, the question whether intensification of noradrenergic transmission constitutes a true possibility for achieving an antidepressant effect has not been settled yet. Most, if not all of the currently available drugs possess manifold pharmacological properties, including NA uptake inhibition, α-adrenolytic, antiserotonergic, antihistaminic and anticholinergic effects. This makes it difficult to determine which of the properties are important for the clinical effect. A kind of 'active placebo', sharing a number of pharmacological effects with true antidepressants, but lacking a property considered crucial according to a particular hypothesis, would therefore be of considerable value. In this paper, we will describe a pair of enantiomers, one of which could serve such a purpose.

Oxaprotiline (C-49802-B-Ba) has been reported to be a highly potent and selective inhibitor of NA uptake in rat heart and brain *in vivo* [16]. It has recently been shown to possess antidepressant effects in man [17, 18]. Oxaprotiline contains a chiral center, and the two optical isomers have been resolved. The structures and absolute configurations (G. Rihs, in preparation) are shown in Fig. 1. The effects of the racemate and its enantiomers on NA and serotonin (5-HT) uptake in rat tissue *in vitro* and *in vivo* and on α_1 and α_2 adrenoceptors *in vitro* are described below.

MATERIALS AND METHODS

Animals. Female Tif:RAIf(SPF) rats (Tierfarm Sisseln, Switzerland) weighing 160–200 g were used if not otherwise stated.

Amine concentrations. For the determination of NA, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA), tissues were homogenized in acidified n-butanol. After addition of n-heptane, the amines

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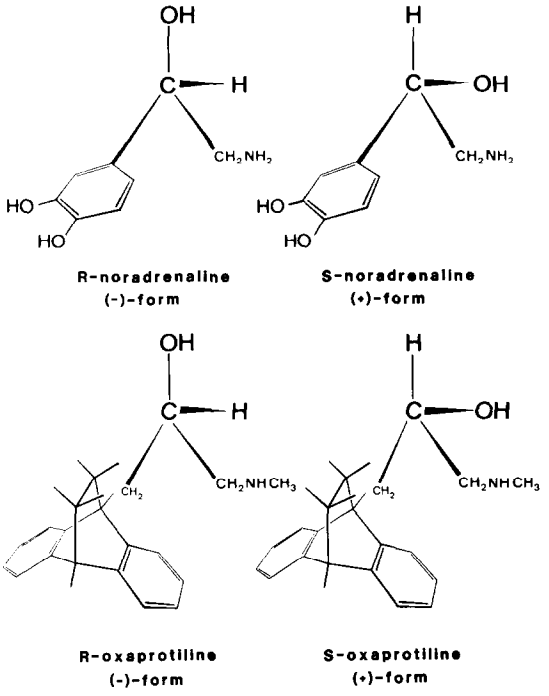

Fig. 1. Absolute configurations of the enantiomers of oxaprotiline and noradrenaline.

were back-extracted into 0.2 N HCl, and 5-HIAA in a subsequent step was extracted into 0.5 M phosphate buffer of pH 7 (a combination of the methods described by Maickel *et al.* [19] and Curzon and Green [20]). The compounds were quantitated fluorometrically [20, 21].

Uptake of [^{3}H]*NA into the rat heart.* 1-[^{3}H]NA (100 μCi/kg, sp. act. 8–10 Ci/mmole, Radiochemical Centre, Amersham, U.K.) was injected 1 hr, or as indicated, after treatment with the drugs to be tested. One hour later, the animals were decapitated, the hearts removed and [^{3}H]NA isolated by alumina adsorption or by butanol extraction as above, and radioactivity counted [22].

Antagonism by 4,α-dimethylmetatyramine (H 77/77) of brain NA and by 4-methyl-α-ethylmetatyramine (H 75/12) of brain 5-HT depletion. The antagonism by drugs of the depleting effects of H 77/77 on brain NA and of H 75/12 on brain 5-HT were used as measures of NA and 5-HT uptake inhibition *in vivo* [23, 24]. The drugs to be tested were administered 30 min before the depletors (obtained from Labkemi AB, Goeteborg, Sweden), which were injected s.c. in doses of 6.25 mg/kg (H 77/77) and 25 mg/kg (H 75/12). The animals were decapitated 4 hr later and whole brain NA and 5-HT determined as described above.

Uptake of [^{3}H]*NA and* [^{3}H]-5-*HT into rat brain synaptosomes* in vitro *and after pretreatment.* The uptake of 1-[^{3}H]NA (sp. act. 4–6 Ci/mmole, New England Nuclear, Boston, MA) and [^{3}H]-5-HT (12.5 Ci/mmole, New England Nuclear) into synaptosomes obtained from the midbrain–diencephalon region of rats was determined as described recently [25]. The concentrations of NA and 5-HT in the incubation media were 10^{-8} and 2.5×10^{-9} M, respectively. The incubation periods were 15 min for [^{3}H]NA and 6 min for [^{3}H]-5-HT. For *ex vivo* experiments, synaptosomes were used from animals which were treated with the drugs to be tested 1 hr prior to decapitation.

Binding of [^{3}H]*prazosin to rat cortical membranes.* Twenty male Tif:RAIf(SPF) rats weighing about 200 g were decapitated, the cerebral cortices rapidly dissected and homogenized in 40 ml of 0.05 M Tris–HCl buffer, pH 7.7. Centrifugation and washing of the membrane fraction was carried out as described by Dooley and Bittiger [26]. The final pellet was suspended in 40 ml of the same buffer; 2 ml aliquots were frozen in liquid propane and stored in liquid nitrogen. Just before use, membranes were thawed quickly at 30–37°, and [^{3}H]prazosin binding was carried out according to Greengrass and Bremner [27], as modified by Dooley and Bittiger [26]. Reactions were carried out in 2 ml 50 mM Tris–HCl, pH 7.4, containing 0.05% ascorbic acid, *ca* 300 μg membrane protein, and 0.25–0.3 nM [^{3}H]prazosin (New England Nuclear, 17 Ci/mmole). Nonspecific binding was defined in the presence of 10^{-6} M prazosin.

Release of [^{3}H]*NA from rat cortical slices by electrical field stimulation.* A slightly modified version of the method of Farnebo and Hamberger [28] was used. Cortical slices from adult male rats were incubated for 30 min in Krebs–Ringer bicarbonate buffer (equilibrated with 5% CO_2 in O_2) at 37° in the presence of 1.5×10^{-7} M 1-[^{3}H]NA (sp. act. 4–6 Ci/mmole, New England Nuclear). The slices were transferred to stimulation chambers and superfused at 37° (1 ml/min) with Krebs–Ringer buffer containing 2×10^{-5} M cocaine to prevent NA uptake. The superfusate was collected in 5-min fractions. After superfusion for 30 min, the slices were stimulated for 2 min in an electrical field (monophasic pulses 2 msec, 3 Hz, 12 mA). The drug to be tested was then added to the superfusion medium and the slices were stimulated again for 2 min, 20 min after the first stimulation. The radioactivity of the fractioned superfusion medium and of the slices (after solubilization in 1 ml Soluene-100®) was measured after addition of 10 ml Instagel® or Aquasol® and Dimilume® scintillator solution, respectively. The stimulation-induced ^{3}H-overflow was calculated by subtracting the estimated spontaneous ^{3}H-overflow from the total ^{3}H-overflow as described by Farnebo and Hamberger [28]. It was expressed as a percentage of the total tritium content in the slices at the onset of each stimulation. The overflow resulting from the first stimulation (S 1) served as a control for the overflow produced by the second (S 2), before which the drug had been added to the superfusion medium.

RESULTS

Acute experiments

Effects of oxaprotiline and its enantiomers on the uptake of [^{3}H]*NA into the rat heart.* After oral administration, (±)-oxaprotiline dose-dependently inhibited the accumulation of [^{3}H]NA into the rat

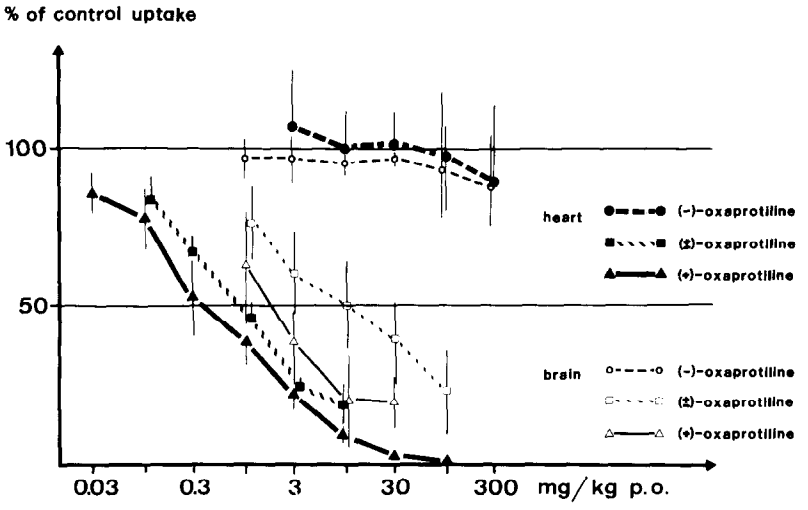

Fig. 2. Effects of oxaprotiline and its enantiomers on NA uptake in rat heart and brain. In the heart experiments, rats were treated with the drugs 1 hr before the injection of [^{3}H]NA, and were decapitated 1 hr later. Four animals were used per group. Data are given as percentage of control uptake ± confidence limits at the 5% level. For the assessment of drug effects on NA uptake in the brain, drugs were administered 30 min before H 77/77 and the brains removed 4 hr later. Five animals were used per group. Percentage uptake inhibition was set equal to percentage reversal of NA depletion [23, 24]. Bars represent confidence limits at the 5% level, calculated according to [33] for equal and according to [34] for different variances.

heart with an ED_{50} of 0.8 mg/kg. The same was observed with its (+)-enantiomer, which had an ED_{50} of 0.35 mg/kg. In contrast, (−)-oxaprotiline was ineffective up to 300 mg/kg (Fig. 2).

The ED_{50}s of (±)-oxaprotiline after subcutaneous and intravenous administration were 1.2 and 0.55 mg/kg, respectively.

Effects on the depletion of rat brain NA induced by H 77/77. The depletion of brain NA by H 77/77 was dose-dependently antagonized by (±)- and (+)-oxaprotiline, with respective ED_{50}s of 10 and 2 mg/kg p.o. The (−)-enantiomer was completely devoid of such activity at doses up to 300 mg/kg p.o. (Fig. 2). Neither drug induced a significant alteration in cerebral NA levels when given alone, at any dose used.

Time-courses of the effects of (±)-oxaprotiline on the uptake of [^{3}H]NA into the rat heart and on the depletion of cerebral NA by H 77/77. The time-course of the effect of (±)-oxaprotiline on [^{3}H]NA uptake into the rat heart at a dose of 30 mg/kg p.o. is shown in Fig. 3. For comparison, the time-courses of the corresponding effects of imipramine (30 mg/kg p.o.) and maprotiline (100 mg/kg p.o.) are also given. The uptake inhibitory effects of oxaprotiline and imipramine had vanished after 72 hr; maprotiline still showed a slight effect at that time. The half-lives of the disappearance of the uptake inhibiting effects of the three compounds, determined graphically from these data, were approximately 20 hr for (±)-oxaprotiline and imipramine and about 30 hr for maprotiline.

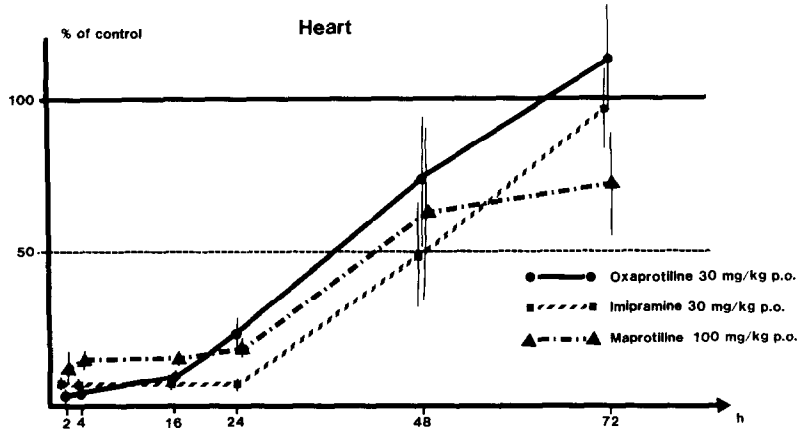

Fig. 3. Time-course of the effect of (±)-oxaprotiline on [^{3}H]NA uptake in the rat heart. The animals received 30 mg/kg p.o. (±)-oxaprotiline or, for comparison, 30 mg/kg p.o. imipramine or 100 mg/kg p.o. maprotiline at the times indicated before the administration of [^{3}H]NA, and were decapitated 1 hr later. Results are given as percentage of controls ± confidence limits at the 5% level ($n = 5$).

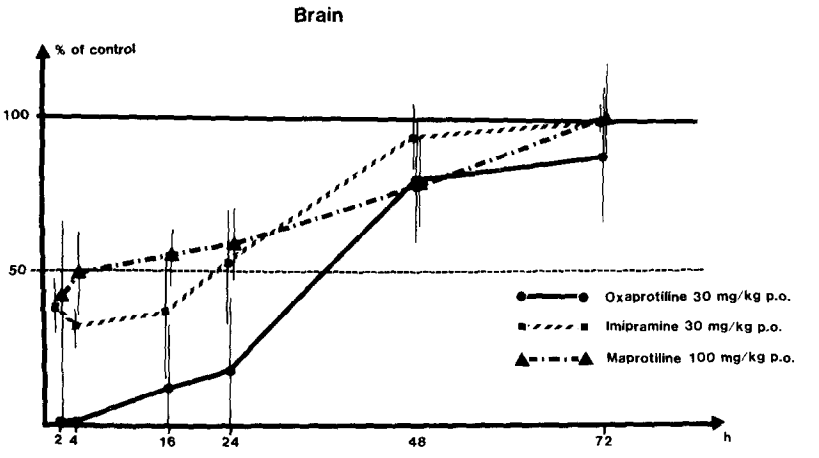

Fig. 4. Time-course of the effect of (±)-oxaprotiline on the depletion of brain NA by H 77/77. The animals received 30 mg/kg p.o. oxaprotiline or, for comparison, 30 mg/kg p.o. imipramine or 100 mg/kg p.o. maprotiline at the times indicated before H 77/77. The animals were decapitated 4 hr later. Data represent percentage uptake inhibition (set equal to percentage reversal of NA depletion) ± confidence limits at the 5% level ($n = 5$).

Table 1. Effects of oxaprotiline and its enantiomers on the uptake of [^{3}H]NA and [^{3}H]-5-HT into rat brain synaptosomes *in vitro*

Treatment	[^{3}H]NA uptake IC_{50} (μM)	[^{3}H]-5-HT uptake IC_{50} (μM)
(±)-Oxaprotiline	0.0046	25
(+)-Oxaprotiline	0.0036	ND
(−)-Oxaprotiline	3	ND
Imipramine	0.046	0.55
Maprotiline	0.046	>100

IC_{50} Values were determined by graphical interpolation from concentration–response curves (spaced by factors of 10) run in duplicates; ND = not detected.

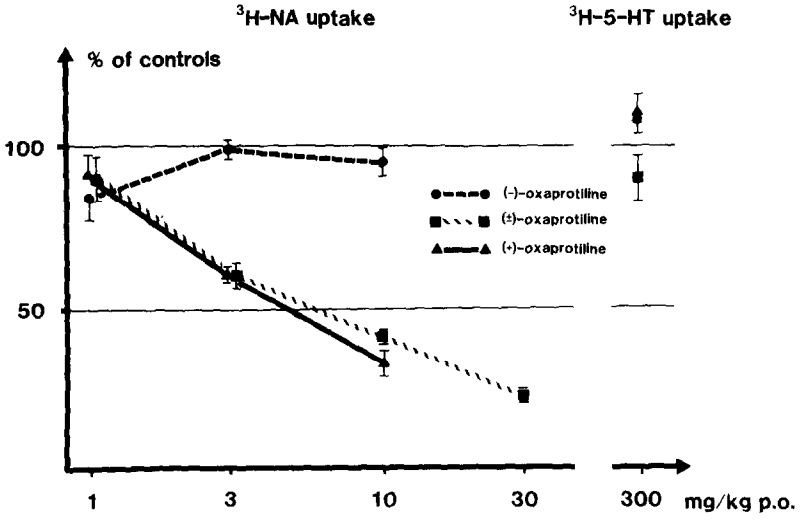

Fig. 5. Effects on the uptake of [^{3}H]NA and [^{3}H]-5-HT into rat brain synaptosomes after pretreatment. Synaptosomes were prepared from brains of rats pretreated for 1 hr. Data represent means ± S.E.M. as percentage of controls ($n = 6$).

The time-course of the effect of (±)-oxaprotiline on the H 77/77 induced depletion of brain NA was investigated with oxaprotiline racemate at a dose of 30 mg/kg p.o. For comparison, corresponding experiments were performed with imipramine (30 mg/kg p.o.) and maprotiline (100 mg/kg p.o.). As shown in Fig. 4, the effects of the three compounds had disappeared 48 hr after treatment. The half-lives of the disappearances were 15 hr for oxaprotiline and imipramine and about 30 hr for maprotiline.

Effects on the uptake of [^{3}H]*NA and* [^{3}H]-5-*HT into rat brain synaptosomes* in vitro. Table 1 contains the IC_{50} values, determined graphically from concentration–response curves, of (±)-oxaprotiline, its enantiomers, and, as reference compounds, imipramine and maprotiline. The racemate of oxaprotiline was about 10 times more potent with respect to inhibition of [^{3}H]NA uptake than imipramine and maprotiline; (+)-oxaprotiline was similarly potent, whereas the (−)-enatiomer was approximately 1000 times weaker. (±)-Oxaprotiline was about 5000 times weaker with respect to [^{3}H]-5-HT uptake than in inhibiting [^{3}H]NA uptake, comparable to maprotiline. The enantiomers were therefore not tested separately.

Effects on the uptake of [^{3}H]*NA and* [^{3}H]-5-*HT into rat brain synaptosomes after pretreatment.* When rats were pretreated for 1 hr with graded doses, (±)-oxaprotiline dose-dependently inhibited the *in vitro* accumulation of [^{3}H]NA into synaptosomes from their brains (ED_{50} = 5.5 mg/kg p.o.). The (+)-enantiomer had approximately the same effect (ED_{50} = 4.5 mg/kg p.o.), whereas the (−)-enantiomer was completely inactive up to a dose of 300 mg/kg p.o. (Fig. 5). Neither the racemate nor the enantiomers inhibited the uptake of [^{3}H]-5-HT into synaptosomes from rats pretreated with 300 mg/kg p.o.

Effects on the depletion of brain 5-HT induced by H 75/12. Neither (±)-oxaprotiline nor its enantiomers were able to reduce the depletion of cerebral 5-HT induced by H 75/12 at doses of 30 mg/kg p.o. (cf. Table 4). The racemate has previously been shown to be inactive in this respect up to a dose of 300 mg/kg p.o. [16].

Effects on the binding of [^{3}H]*prazosin to rat cortical membranes.* Oxaprotiline racemate was about 2-fold less active than imipramine and 30-fold less active than phentolamine in displacing [^{3}H]prazosin from its binding sites. (+)-Oxaprotiline was about 6-fold less active than the (−)-enantiomer (Table 5). Since prazosin is thought to bind specifically to α_1 adrenoceptors in the brain [27, 29], this indicates that the α_1 antagonistic properties of the racemate of oxaprotiline are comparable to those of imipramine, and that the (−)-enantiomer is more potent in this respect than the (+)-form.

Effect of oxaprotiline racemate on the field-stimulated release of [^{3}H]*NA from rat cortical slices.* (±)-Oxaprotiline at concentrations of 10^{-6} and 10^{-5} M did not exhibit any effect on impulse-mediated release of radioactivity (^{3}H-overflow) from cortical slices prelabelled with [^{3}H]NA (Table 5), but caused some spontaneous release at the higher concentration. In view of this lack of effect, the two enantiomers were not tested separately.

Imipramine caused a 50% increase in ^{3}H-overflow at concentrations about 40 times higher than those which displaced [^{3}H]prazosin by 50%; phentolamine did so in concentrations comparable to those which affected [^{3}H]prazosin binding.

Since these experiments were carried out in the presence of cocaine, the increases in ^{3}H-overflow are likely to reflect α_2 antagonistic properties of the compounds.

Repeated administration

Effects on [^{3}H]*NA uptake into the rat heart.* Rats were treated once or once daily with (±)-oxaprotiline and the enantiomers. For the racemate and the active enantiomer, a dose below and a dose close to or somewhat above the ED_{50} were chosen to be able to detect possible cumulative effects or attenuations. The inactive (−)-enantiomer was administered in high dosages to detect possible late effects, e.g. due to racemization in the organism.

The effect of (±)-oxaprotiline on [^{3}H]NA uptake

Table 2. Effects of oxaprotiline and its enantiomers on the uptake of [^{3}H]NA into the rat heart after single and 10 daily administrations

Treatment	mg/kg p.o.	% of control uptake: Single administration	% of control uptake: 10 daily administrations
(±)-Oxaprotiline	0.1	71 ± 5* (5)	73 ± 8 (5)
	1	39 ± 6† (5)	45 ± 4† (4)
(+)-Oxaprotiline	0.1	74 ± 2 (5)	60 ± 2†‡ (4)
	1	21 ± 4† (5)	27 ± 3† (4)
(−)-Oxaprotiline	10	104 ± 9 (5)	111 ± 13 (5)
	30	120 ± 8 (5)	105 ± 7 (5)
	100	99 ± 7 (5)	83 ± 8 (5)

* $P < 0.05$, † $P < 0.01$ vs controls (Dunnett's *t*-test).

‡ $P < 0.001$ vs single administration (Student's *t*-test).

The animals were treated once or once daily for 10 days. One hour after the last treatment, [^{3}H]NA (100 μCi/kg) was injected into the tail vein and the animals sacrificed 1 hr thereafter. [^{3}H]NA was extracted from the hearts and counted. Data are given as percentages of controls ± S.E.M. The number of animals per group is indicated in brackets. Endogeneous NA levels were also determined; there was no significant difference with respect to controls in any of the treated groups.

Table 3. Effects of oxaprotiline and its enantiomers on the depletion of cerebral NA by H 77/77 after single and 10 daily administrations

Treatment (mg/kg p.o. × days)		NA concentration (ng/g) − H 77/77	NA concentration (ng/g) + H 77/77	Percentage uptake inhibition
Controls		321 ± 11 [9]	159 ± 8 [5]	0
(±)-Oxaprotiline	3 × 1	326 ± 3 [5]	208 ± 13 [4]	29 (14–43)
	3 × 10	337 ± 7 [5]	236 ± 7 [5]	43 (32–54)
(+)-Oxaprotiline	3 × 1	324 ± 12 [5]	257 ± 13 [5]	59 (41–79)
	3 × 10	344 ± 5 [5]	246 ± 6 [4]	47 (37–57)
(−)-Oxaprotiline	30 × 1	353 ± 12 [5]	144 ± 4 [5]	−8 (−23– 6)
	30 × 10	367 ± 12 [5]	162 ± 3 [5]	1 (−12–13)
Imipramine	10 × 1		245 ± 9 [5]	53 (33–72)

Rats were treated once or once daily for 10 days with oxaprotiline or its enantiomers. Thirty minutes after the last administration, H 77/77 (6.25 mg/kg s.c.) was injected and the animals decapitated 4 hr later. Whole brain NA concentrations are given in ng/g ± S.E.M.; the number of animals per group is indicated in square brackets. In the last column, uptake inhibition is calculated according to Carlsson *et al.* [23, 24]; in brackets, the confidence limits at the 5% level, calculated according to Fieller [33] for equal and to Chakravarti [34] for unequal variances.

Whole brain dopamine levels were also determined in the groups without H 77/77 treatment. No significant changes (Dunnett's *t*-test) were observed.

into the rat heart was similar in extent, whether the drug was given acutely or 10 times once daily (Table 2). With (+)-oxaprotiline, the effect of the lower dose was slightly more pronounced after repeated than after acute administration, and this was statistically significant at the 0.1% level. No such difference was noted with the higher dose. (−)-Oxaprotiline was ineffective also after 10 daily administrations even at the high doses used (up to 100 mg/kg p.o.).

Antagonism of rat brain NA depletion by H 77/77. A similar experiment was carried out to investigate the effects of oxaprotiline and its enantiomers on NA uptake in the brain after repeated administration (Table 3). An acute dose of imipramine (10 mg/kg p.o.) was included in this experiment for reference purposes. The dose of (±)- and (+)-oxaprotiline (3 mg/kg p.o.) was chosen close to the ED_{50}s determined previously (Fig. 2), and (−)-oxaprotiline was given at a 10 times higher dose.

(±)-Oxaprotiline was slightly less effective acutely than if administered 10 times; this difference was, however, not statistically significant. The (+)-enantiomer showed the same effect whether given acutely or repeatedly, and the (−)-enantiomer remained inactive after 10 daily treatments (Table 3).

None of the compounds changed NA concentrations, neither after acute nor after repeated administration.

Antagonism of rat brain 5-HT depletion by H 75/12. At a dose of 30 mg/kg p.o., neither the racemate nor the enantiomers caused a reduction of the depleting effect of H 75/12, whether the drugs were given acutely or repeatedly. This indicates that the compounds do not affect 5-HT uptake.

Moreover, no change in 5-HT levels was observed after acute or repeated administration. Neither were there significant alterations of 5-HIAA concentrations; tendencies towards reductions were noted, however, after repeated administration of the race-

Table 4. Effects of oxaprotiline and its enantiomers on the H 75/12-induced 5-HT depletion and on 5-HT and 5-HIAA concentrations in rat brain after single and repeated treatment

Treatment (mg/kg p.o. × days)		5-HT (ng/g) Without H 75/12	5-HT (ng/g) With H 75/12	Per cent 5-HT uptake inhibition	5-HIAA (ng/g)
Controls		365 ± 14 [5]	183 ± 6* [5]		243 ± 7 [5]
(±)-Oxaprotiline	30 × 1	349 ± 9 [5]	212 ± 5 [4]	17 (4–30)	232 ± 6 [5]
	30 × 10	350 ± 7 [4]	198 ± 15 [5]	9 (−12–26)	217 ± 7 [5]
(+)-Oxaprotiline	30 × 1	346 ± 11 [5]	190 ± 9 [5]	4 (−14–20)	250 ± 16 [5]
	30 × 10	336 ± 7 [4]	197 ± 7 [5]	9 (−4–21)	215 ± 13 [5]
(−)-Oxaprotiline	30 × 1	367 ± 8 [5]	185 ± 5 [5]	1 (−10–11)	249 ± 9 [5]
	30 × 10	369 ± 5 [3]	178 ± 2 [4]	−3 (−12– 6)	253 ± 9 [5]

* $P < 0.01$ vs controls (Dunnett's *t*-test). The three compounds were given at the dose of 30 mg/kg p.o. once or once daily for 10 days. H 75/12 (25 mg/kg s.c.) was administered 30 min after the last treatment and the animals decapitated 4 hr later. The values represent means ± S.E.M.; the number of animals used per group is indicated in square brackets. Uptake inhibition was calculated as percentage reversal of the depletion induced by H 75/12 [23, 24]. Confidence limits at the 5% level (in brackets) were calculated according to Fieller [33] for equal or to Chakravarti [34] for unequal variances. None of the values of the combined groups was significantly different from H 75/12 alone. None of the values of 5-HT or 5-HIAA of the groups treated with the drugs alone was significantly different from controls (Dunnett's *t*-test).

mate and the (+)-form, but not of (−)-oxaprotiline (Table 4).

DISCUSSION

Oxaprotiline is a highly potent and selective inhibitor of NA uptake *in vitro* and *in vivo*, as shown by the above experiments in the rat. In synaptosomes *in vitro*, it is 5000 times more potent as an inhibitor of NA than of 5-HT uptake, and in rat brain *in vivo*, no 5-HT uptake inhibiting effects could be detected at doses 100 times higher than the ED_{50} for NA uptake inhibition (see also [16]). As a NA uptake inhibitor *in vivo*, compared under identical conditions, it is as potent as desipramine in the brain, and several-fold more potent than the latter in the heart [30].

The duration of action after a high dose (30 mg/kg p.o.), corresponding to about 30 times the ED_{50} in the heart and about 3 times that in the brain, was between 48 and 72 hr in the former and about 48 hr in the latter. This is similar to what is observed with imipramine or maprotiline, and means that a once daily administration is a valid procedure for studies of the effects of repeated treatment in the rat. After 10 daily administrations, the selectivity of oxaprotiline with respect to NA uptake inhibition was maintained: no 5-HT uptake inhibiting effects were seen at a daily dose of 30 mg/kg p.o. There was also no cumulation of the NA uptake inhibitory effect with doses close to the respective ED_{50}s in heart and brain.

The study of the effects of the enantiomers of oxaprotiline showed the NA uptake inhibiting effects to be confined entirely to the (+)- or S-enantiomer. The (−)- or R form was about 1000 times less active than the (+)-form in synaptosomes *in vitro*. This minimal effect can probably by ascribed to traces of the (+)-form remaining from the separation of the enantiomers. *In vivo*, (−)-oxaprotiline was devoid of NA uptake inhibiting properties in doses exceeding the ED_{50}s of the (+)-form by a factor of about 100 in both heart and brain.

After 10 daily treatments, the effect of the (+)-enantiomer on NA uptake did not cumulate in heart or brain, nor did it affect 5-HT uptake at higher doses, in agreement with what had been found previously with the racemate. The (−)-form remained inactive after repeated treatment, both with respect to NA as well as 5-HT uptake, suggesting that no racemization occurred in the organism during the treatment period. Both (±)- and (+)-oxaprotiline, but not the (−)-form, exhibited a tendency to lower 5-HIAA concentrations after repeated treatment. Although these effects were not statistically significant, they might indicate an interference of these compounds with 5-HT transmission after repeated treatment. Since the (−)-form was ineffective and both enantiomers appear to lack antiserotonergic effects [32], one might suspect a causal relationship with the NA uptake inhibiting properties of (±)- and (+)-oxaprotiline. In any case, more detailed studies of the phenomenon are necessary.

The lack of NA uptake inhibiting properties of the (−)-form of oxaprotiline does not mean that it is simply an inactive compound, however. For instance, it possesses antihistaminic and antiaggressive properties to a similar extent as the (+)-form [31, 32]. Moreover, its α_1 antagonistic properties are stronger than those of the (+)-enantiomer, and comparable to those of imipramine. The α_2 antagonistic effects of both enantiomers are in all probability negligible, since the racemate did not exhibit any at very high concentrations.

In fact, the most clearcut difference between the two enantiomers detected hitherto consists in their effect on NA uptake. It is of interest to note that the absolute configuration of the inactive form corresponds to that of (−)-NA, the naturally occurring enantiomer of the catecholamine (see Fig. 1). There is no explanation available at present for this phenomenon.

Oxaprotiline has been found to be an effective antidepressant with remarkably little side effects in the two studies hitherto published [17, 18]. This impression is backed up by the unpublished material presently available (approximately 300 cases). It is therefore of considerable interest to investigate the relevance of NA uptake inhibition by comparing the antidepressant action of the two enantiomers in patients. In other words, such a comparison would be an important test for the validity of the catecholamine hypothesis of depression. Moreover, this pair of enantiomers can also be expected to be useful in animal studies, especially in chronic experiments. For example, it permits the determination of those effects of antidepressants which are ultimately due to NA uptake inhibition and which are related to

Table 5. Effects of oxaprotiline and its enantiomers on the binding of [^{3}H]prazosin to cortical membranes and on impulse-mediated release of [^{3}H]NA from cortical slices of the rat

Treatment	[^{3}H]Prazosin binding (IC_{50}, M)	[^{3}H]NA release (EC_{50}, M)
(±)-Oxaprotiline	10^{-6}	0 at 10^{-6} and 10^{-5}
(+)-Oxaprotiline	3×10^{-6}	
(−)-Oxaprotiline	5×10^{-7}	
Imipramine	4×10^{-7}	1.6×10^{-5}
Phentolamine	3×10^{-8}	1.6×10^{-8}

IC_{50} and EC_{50} (concentration at which [^{3}H]overflow is increased by 50%) were determined by graphical interpolation.

The stimulation-induced ^{3}H-overflow in the presence of 2×10^{-5} M cocaine, but without test drug (S_1; see Methods) was between 4.4 and 6.1 per cent of the radioactivity present in the slices at the onset of the first stimulation period (S_1).

other properties. To serve such a purpose, however, a complete characterization of the pharmacological properties of the two compounds is mandatory.

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