Table 2. Effect of representative concentrations on free fraction (% unbound) at initial (pre-dialysis) total plasma concentrations of unlabelled drug from 10–10 000 ng ml^{-1}. (n = 10).

Drug	10	100	600	1000	4000	10000	Mean (± s.e.)
Diazepam	1·58	1·53	1·64	1·60	1·63	1·62	1·58 (± 0·01)
Desmethyldiazepam	3·53	3·51	3·72	3·25	3·54	3·64	3·47 (± 0·05)
Temazepam	3·59	3·40	3·97	3·79	3·68	3·50	3·62 (± 0·07)
Midazolam	3·71	3·72	3·62	3·61	3·55	3·58	3·66 (± 0·03)
Oxazepam	5·55	5·22	4·94	4·73	4·78	5·05	5·12 (± 0·08)
Lorazepam	9·85	9·76	9·61	9·78	9·49	9·77	9·74 (± 0·04)
Clobazam	16·8	16·3	16·2	16·9	16·4	18·1	16·9 (± 0·2)
Tiazolam	22·6	23·4	21·5	21·6	22·1	21·1	22·1 (± 0·28)
Flunitrazepam	21·6	22·6	20·2	21·4	22·1	23·1	22·5 (± 0·4)
Alprazolam	34·0	32·6	28·4	31·9	28·9	32·0	31·6 (± 0·6)

although in some cases of massive overdosage levels in this range have been reported (Greenblatt et al 1978). The findings suggest that concentration-dependent plasma protein binding should not complicate the interpretation of pharmacokinetic studies of benzodiazepines following usual therapeutic doses.

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(+)-Oxaprotiline but not (−)-oxaprotiline given chronically potentiates the aggressive behaviour induced by clonidine

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Long-term, but not acute, treatment with antidepressants (tricyclics, maprotiline, nisoxetine, mianserin, iprindole, zimelidine, levomepromazine, thioridazine) potentiates aggression induced by clonidine in mice (Maj et al 1980, 1981, 1982). Clonidine aggression is realized by α_1-adrenergic stimulation (Morpurgo 1968; Ozawa et al 1975; Maj et al 1981), then its potentiation by antidepressants can be attributed to the noradrenergic mechanism. To support such a hypothesis we have examined (+)- and (−)-oxaprotiline, of which only the (+)-form inhibits noradrenaline uptake, evokes the typical antidepressant pharmacological effects, and, given chronically, induces β-adrenergic subsensitivity (Bittiger et al 1981; Mishra et al 1981).

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Method

The experiments were carried out on male Albino Swiss mice (20–30 g) housed in groups and having free access to food and water throughout the experiment. (+)- and (−)-Oxaprotiline (hydrochloride, 10 mg kg^{-1}) or 0·9% NaCl (saline) were injected i.p. twice a day for 14 days. Clonidine (hydrochloride, 20 mg kg^{-1} i.p. in saline) was given 2 h after the last dose of oxaprotiline. Immediately thereafter groups of four mice each were placed together in glass cylinders and the number of biting attacks was counted for 1 h (Ozawa et al 1975). The acute experiments (single dose of (+)- or (−)-oxaprotiline) were performed in a similar manner. The dose of clonidine was choosen on the basis of previous experiments (Maj et al 1980, 1981) and was administered 2 h after oxaprotiline. Both drugs were dissolved in saline. In each (acute or chronic) experimental group

Table 1. The effects of (+)- and (−)-oxaprotiline on clonidine-induced aggression in mice. Acute—all mice received single injections of saline or oxaprotiline (10 mg kg^{-1} i.p.) Chronic—all mice received injections of saline or oxaprotiline (10 mg kg^{-1} i.p.) twice a day for 14 days. Clonidine hydrochloride, 20 mg kg^{-1}, was given i.p. 2 h after a single dose (acute experiment) or after the last dose (chronic experiment) of saline or drug. The means represent the number of biting attacks among 4 mice within 1 h after clonidine. In each group there were 32 mice (8 groups of 4). Statistical significance was assessed by the Student's *t*-test (oxaprotiline + clonidine versus saline + clonidine).

	Number of biting attacks after clonidine			
	Acute		Chronic	
Pretreatment	mean±s.e.m.	%	mean±s.e.m.	%
Saline	55·6±9·5	100	47·5± 8·1	100
(+)-Oxaprotiline	48·3±7·6 n.s.	86·7	135·1±14·8 $P<0.001$	284·5
(−)-Oxaprotiline	57·4±9·7 n.s.	103·1	70·8± 8·0 n.s.	148·9

there were 32 mice (8 groups of 4). The statistical significance was assessed by Student's *t*-test.

Results

(+)-Oxaprotiline, given in a single (10 mg kg^{-1}) dose did not influence clonidine aggression (Table 1). Repeated treatment markedly intensified the aggressive behaviour. (−)-Oxaprotiline (10 mg kg^{-1}) given either acutely or chronically did not modify clonidine aggression. Both enantiomers given alone acutely or chronically did not induce aggressive behaviour (data not given).

Discussion

We have previously observed that the secondary increase of the response to clonidine might also be induced by a primary blockade of α_1-adrenoceptors. However, neither of the enantiomers of oxaprotiline have any such activity in either the hind limb flexor reflex model (evaluated according to the method described by Maj et al 1976) or in the blood pressure test in pithed rat (data not shown).

Thus, of the two enantiomers of oxaprotiline only the (+)-form, given chronically, but not acutely, potentiates (as do two other selective noradrenaline uptake inhibitors, maprotiline and nisoxetine, Maj et al 1981, 1982) clonidine aggression and inhibits noradrenaline uptake. A similar action of imipramine and amitriptyline which inhibit the uptake of noradrenaline and 5-hydroxytryptamine (5-HT) may be associated with the inhibition of noradrenaline uptake, since the selective 5-HT uptake inhibitors, citalopram, fluoxetine, fluvoxamine, do not intensify clonidine aggression (Maj et al 1980, 1981, 1982). Therefore, the present results offer further argument for the hypothesis that chronic administration of antidepressants leads to an increased responsiveness of the α_1-adrenoceptors for which clonidine is an agonist.

A similar conclusion was also drawn from the findings that the chronic treatment with a number of antidepressants, but not fluoxetine, intensifies the response to noradrenaline in the lateral geniculate nucleus or in the facial nucleus (Menkes et al 1980; Menkes & Aghajanian 1981).

According to Sulser and his colleagues (1979) a variety of antidepressants given chronically induces β-adrenergic subsensitivity. There is a good agreement between those results and our findings with the clonidine aggression model. In both cases many typical and atypical antidepressants are active whereas fluoxetine is ineffective. The example of nisoxetine, which modifies the responsiveness of the cyclic AMP generating system but not the density of β-adrenoceptors (Sulser 1979) and at the same time intensifies clonidine aggression, shows that agreement concerns primarily the first effect. Whether and what kind of relation exists between the increased responsiveness of α_1-adrenoceptors and the decreased responsiveness of β-adrenoceptors remains to be explained.

The increased responsiveness of the α_1-adrenergic system found here, and previously, may be responsible for the therapeutic effect of antidepressant drugs.

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