Pharmacology Biochemistry & Behavior, Vol. 19, pp. 719–723, 1983. © Ankho International Inc. Printed in the U.S.A.

The Effects of Single and Repeated Doses of Maprotiline, Oxaprotiline and its Enantiomers on Foot-Shock Induced Fighting in Rats

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Received 14 February 1983

MOGILNICKA, E., C. G. BOISSARD, P. C. WALDMEIER AND A. DELINI-STULA. *The effects of single and repeated doses of maprotiline, oxaprotiline and its enantiomers on foot-shock induced fighting in rats.* PHARMACOL BIOCHEM BEHAV **19**(4) 719–723, 1983.—Foot-shock induced fighting behavior (SIF) in rats was tested after single and repeated dose-treatment (10 mg/kg IP twice daily for 10 days) of maprotiline, oxaprotiline and of (+)- and (−)-enantiomers of oxaprotiline. Marked facilitation of SIF was observed after repeated but not single administration of all drugs including the NA-uptake inactive (−)-enantiomer of oxaprotiline. No enhancement of SIF was seen after multiple dose-treatment with promethazine, an antihistaminic, or atropine. The mechanism of the facilitation of SIF induced by antidepressants maprotiline and oxaprotiline as well as by its enantiomers is unclear. The clear-cut dissociation of the effect of (+)- and (−)-oxaprotiline on the rate of NA-disappearance, but their similar enhancing effect on SIF challenges the assumption of a primary importance of central NA-system in this behavior. By contrast, the increase in jumping behavior recorded additionally to SIF, seems to be a great extent dependent on NA-uptake inhibiting properties of tested drugs.

Shock-induced fighting Noradrenaline Jumping behavior Antidepressants Chronic treatment Maprotiline Oxaprotiline (+)-Oxaprotiline (−)-Oxaprotiline

IN CONTRAST to the well established inhibitory effects of antidepressants (ADs) on predatory aggression (muricide behaviour), their influence on affective types of aggressiveness produced by isolation or footshock-stimulation appears to be variable. Thus for instance after single doses, certain ADs do not change affective aggressive reactions or slightly increase it, others by contrast inhibit it in a dose-dependent fashion. Very little is at present known about the effect of long-term treatment with ADs on such affective types of aggressiveness and only few reports dealing with this problem have been published until now [5, 13, 17]. In view of the fact that chronic effects of ADs in animals may be of greater relevance for their clinical antidepressant activity than those observed after single doses, we found of interest to study the effects of maprotiline (MAP), oxaprotiline (±) OX and of its (+) and (−)-enantiomer on shock induced fighting (SIF) in the rat. This particular aggression model has been selected for the study because of the suspected role of noradrenaline (NA) in eliciting this type of affective response [6].

In recent studies it has been shown that single doses of maprotiline exert inhibitory effects on foot-shock-induced fighting in mice, but not in rats [3,9]. (±)OX, (a hydroxyderivative of MAP) showed similar antiaggressive properties in mice as MAP [2], however, its effects in rats have not yet been reported. Also, possible influence of repetitive treatment with these two drugs on SIF has not yet been studied. In this respect, particularly interesting was to test the effects of (+) and (−)-enantiomer of (±)OX since its highly selective NA-uptake inhibiting properties are stereoselective and entirely confined to the (+)-form [21]. Promethazine, an antihistaminic, and atropine were tested for comparison. Moreover, studies of the rate of NA-disappearance in the rat brain after repeated treatment with (±)OX and its enantiomers were also carried out in order to clarify a possible role of effects thereon in the SIF facilitating properties of (±)OX and its enantiomers.

METHOD

Male albino rats (Tif: RAI f (SPF), Tierfarm Sisseln, Switzerland) weighing 170–190 g at the beginning of the treatment were housed in groups of 10 animals per cage

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under standard laboratory conditions (a continuous 12 hr light-dark cycle, constant humidity and temperature) with free access to food and water. All rats were allowed one day of adaptation to the laboratory in which the tests were performed.

Experiment 1

Rats (n=20 per dose) were treated with one single dose (10 mg/kg) of MAP, (±)OX, (+)OX or (−)OX. Pairs of rats were selected at random from each treatment group and placed 2 hr after the administration of the drug in experimental chambers in which they were subjected to mild electric shocks delivered by a shock scrambler (Ruedin LAS 8010) through an electrifiable grid-floor (see later).

Experiment 2

Animals received twice a day injections of vehicle or 10 mg/kg of drugs for 10 consecutive days (chronic experiment); or were injected twice daily with vehicle for 9 consecutive days. On the 10th day they received a single dose of the tested drug (acute experiment). Thereafter, they were subjected to the test as in Experiment 1.

Experiment 3

Animals were treated twice daily with either vehicle, promethazine (10 mg/kg) or atropine sulphate (5 mg/kg) for 10 consecutive days.

SIF-Procedure

Two hours after the last injection of the vehicle or drugs, pairs of rats (vehicle-vehicle, drug-drug), each rat from different home cage, were placed in an experimental shock-chamber (24×24×42 cm). After 1-min adaptation period, fighting was induced by mild electric foot-shock. A constant current, every 3 sec for 5 min (1 mA intensity and 0.5 sec duration), was delivered by a shock-scrambler (Ruedin LAS 8010) through an electrificable grid-floor. The latency to the onset of the first attack, the number of attacks, and the duration of fighting were recorded during 5-min period. An attack was scored when an animal, in response to shock assumed an upright posture and faced its partner with forepaws in touch. The duration of fighting was defined as total time the rat spent in an upright position during 5 min of exposure to shock. In addition to fighting, the number of individual jumping episodes occurring in fighting-free intervals was counted.

Statistics

The results were statistically analysed by the Mann-Whitney U-test.

Drugs

Hydrochloride salts of MAP, (±)OX and its (+) and (−)-enantiomers (Ciba-Geigy), atropine sulphate (Fluka) and promethazine hydrochloride (Rhône-Poulenc) were injected intraperitoneally as aqueous solutions in a volume of 2 ml/kg. The doses of drugs refer to the forms used.

Biochemical Methods

Determination of the disappearance of endogenous NA in rat cortex after tyrosine hydroxylase inhibition. Groups of 5 females Tif: RAIF (SPF) rats (Tierfarm Sisseln, Switzerland) weighing 180–200 g at the beginning of the treatment were treated with (±)OX (10 mg/kg PO) or (+)- or (−)OX (5 mg/kg PO) once or once daily for 2, 4 or 10 days. Two hours after the last treatment, they received α-methyl-p-tyrosine methylester HCl (H 44/68, Labkemi AB, Gothenburg, Sweden; 250 mg/kg IP) and were decapitated 4 hr later. Brains were dissected, and cortices homogenized in acidified n-butanol. NA was extracted after addition of n-heptane into 0.2 M HCl essentially as described by Maickel *et al.* [8]. Fluorometric estimation of the catecholamine was performed according to Waldmeier *et al.* [22]. Statistical calculations were performed by means of Dunnett's test.

RESULTS

None of the investigated substances given acutely (10 mg/kg) changed the quality or frequency of fighting in comparison to respective control. Thus, in experiments, where the animals received only one injection of vehicle or one dose of drugs (Experiment 1), the mean number of fighting episodes during 5 min of exposure was about 30–40 and the average duration of fighting about 80–160 sec (Table 1).

In the experiment (Experiment 2) in which the animals were handled and treated with vehicle prior to the single dose of drugs, the fighting episodes induced by shocks were very sparse (3–7/5 min) and short-lasting (5–18 sec) both in controls (vehicle-vehicle) and drug-treated (vehicle-drug) groups (Table 1). Generally, these rats behaved differently from the rats which received only single injections and no previous handling. They showed longer latency to the onset of the first attack and became passive shortly (1 min) after shock delivery; they did not fight or try to escape.

By contrast, reptitive administration of 10 mg/kg MAP, OX and its both enantiomers (twice daily injections for 10 days) produced an enhanced aggressive response to foot-shock in that the number of fighting episodes was clearly higher, duration of attacks longer and the latency to the first attack remarkably shorter by comparison to the parallel and identically handled controls. The difference between these drug and control groups (Control 2) was highly significant. These drug-treated animals were, however, in respect to their responsiveness to shock not significantly different from the animals which have not experienced repetitive handling beforehand (Control 1).

As shown in Table 1, in the Experiment 2, the number of jumps was markedly increased by comparison to controls either in rats receiving chronic saline plus single dose of (±)OX or (+)OX or in rats receiving multiple doses of these two substances. MAP and (−)OX also increased jumping frequency, but markedly less than either (±)OX or (+)-enantiomer. After subchronic treatment, the increase of jumping produced by (±)OX and (+)OX was significantly higher ($p<0.01$) than that observed after (−)OX.

Chronic pretreatment with promethazine and atropine slightly and non-significantly reduced fighting. Latency to the first attack as well as jumping behavior was also not significantly changed (median values, duration: control=11, promethazine=3, atropine=4; fighting frequency: control=4.5, promethazine=61, atropine=88.5; jumping frequency: control=6, promethazine=8, atropine=6).

Biochemical Results

Treatment with either (±)OX or the enantiomers did not alter the endogenous concentration of NA in the rat cortex, irrespective of the duration of the treatment (results not

TABLE 1

THE INFLUENCE OF ACUTE AND REPETITIVE TREATMENT WITH MAPROTILINE, (±) OXAPROTILINE AND ITS ENANTIOMERS ON THE FOOTSHOCK-INDUCED FIGHTING IN RATS

	Fighting Behavior median values			Jumping median values	
Treatment	Latency (sec)	Duration (sec)	Frequency (n fights)	Frequency (n jumps)	n
Experiment 1					
Acute‡					
Vehicle (Control 1)	21.5	99	42	6	(20)
Maprotiline	18.5	158	56	7	(10)
(+) Oxaprotiline	26.5	114	43	8	(10)
(±) Oxaprotiline	41	78	31	16.5	(10)
(−) Oxaprotiline	24.5	93	37	4.5	(10)
Experiment 2					
Acute§					
Vehicle (Control 2)	70	5.5	3	3	(30)
Maprotiline	86.5	18.0	7.5	18.0*	(10)
(−) Oxaprotiline	70	4.5	2	42.0*	(10)
(±) Oxaprotiline	79.5	11.5	3	35.5*	(10)
(+) Oxaprotiline	101.5	11.5	6	18.0*	(10)
Chronic					
Maprotiline	21.5*	133*	49.5*	14*	(19)
(−) Oxaprotiline	18.5*	122*	45.0*	36*	(20)
(±) Oxaprotiline	15.0*	124.5*	49.5*	27.5*	(20)
(+) Oxaprotiline	14.5*	137*	50.5*	11.0*†	(20)

All of the compounds were administered in a single dose of 10 mg/kg IP or twice daily for 10 consecutive days. The last dose was administered 2 hr before the test.

All values are expressed as medians, n = number of pairs.

‡Single injections of the drugs or vehicle in previously non-handled animals.

§Single injections of the drugs in animals previously handled and treated with ph. saline.

*$p < 0.01$ by comparison to corresponding controls, Mann-Whitney-U-test.

†$p < 0.01$ by comparison to (−) oxaprotiline or to (+) oxaprotiline.

shown). None of the compounds affected the disappearance of NA in this area after acute treatment. However, repeated administration of the racemate and the (+)-enantiomer significantly enhanced the disappearance of the catecholamine; in contrast, the (−)-enantiomer was inactive in this respect (Fig. 1). With the racemate, in the experiment in which the drug was given acutely and for 2 or 4 days respectively, it became evident that this increase in NA utilization occurred already after the second administration, and seemed to be slightly enhanced after the fourth. In the experiment with the enantiomers, in which the compounds were administered acutely or for 4 or 10 days, respectively, it was noted that the enhancement of NA disappearance observed after 4 days treatment was not further altered after 10 days, indicating that a stable situation with respect to the influence on NA utilisation has been reached after this time.

DISCUSSION

The present study shows that chronic treatment with tetracyclic ADs, MAP, and (+)OX, as well as with its (+)- and (−)-enantiomer facilitates SIF.

Single doses of these drugs, irrespectively of the pretreatment conditions, did not influence SIF.

Increased SIF was shown after repeated doses of desipramine [5,17] as well as after imipramine, amitriptyline, mianserine and iprindole [5,13]. The fact that MAP and (+)OX, drugs having antidepressant activity [7,18], also behave in SIF-test similarly to other ADS, supports the assumption, that facilitation of SIF is a common property of chronically administered ADs. The mechanism of this action is not clear. However, a significant role of the NA-ergic system in this phenomenon is generally suspected. Eichelman and Barchas [5] have related the enhancement of SIF to an increased NA turnover after chronic AD treatment. Since in contrast to (±)OX and (+)OX, chronic treatment with (−)OX does not produce an increase in NA-disappearance in the rat cortex (Fig. 1), this mechanism of action seems highly unlikely. Other authors [13] suggested the relationship between possible changes in the central α-adrenergic receptor sensitivity and SIF. It is known indeed, that (−)OX has α_1-antagonistic properties, in common with (±)OX and (+)OX. These are even stronger than those of the racemate and the (+)-form and comparable to those of imipramine [21]. Therefore, one could speculate that chronic treatment with (−)OX may lead to α_1-adrenoceptor supersensitivity and that this effect is responsible for the observed enhancement of SIF. α_1-Adrenoceptor supersensitivity was found after long-term

treatment with imipramine and amitriptyline [10,20]. However, preliminary estimations by means of ^{3}H-WB-4101 binding assay indicated that neither (±)OX, (+)OX nor (−)OX, given chronically change the sensitivity of α_1-adrenoceptors in the rat cortex (K. Hauser, personal communication).

The role of α_2-adrenoceptors in the mediation of SIF is not yet established. Stimulation of those receptors by small doses of the α_2-agonist clonidine has been, however, reported to inhibit SIF [19]. Speculatively, therefore it could be expected that the subsensitivity of α_2-adrenoceptors may result in an enhancement of SIF. However, at present there is no evidence that α_2-subsensitivity develops—after multiple dose treatment with (−)OX [4]. This is in contrast to MAP, (±)OX and (+)OX which under similar treatment conditions clearly change the α_2-adrenoceptor mediated responses. It is also not likely that β-adrenoceptor subsensitivity is the cause of enhanced SIF after the treatment with (−)OX. There are at least two reasons for this assumption: first, after chronic treatment with (−)OX no changes in the ^{3}H-DHA-binding characteristics or in the formation of cAMP in response to NA in the rat cerebral cortex were observed [1, 4, 11], second, facilitation of SIF was observed in rats after the treatments inducing β-adrenoceptor supersensitivity [6,12].

In discussing the possible mechanisms implicated in the enhanced SIF response it should be mentioned that the facilitator roles of cholinergic [16] and histaminergic systems were recently postulated [14]. Since (±)OX and its enantiomers have rather strong antihistaminic properties and, although weak, anticholinergic effects [2,4], changes in the reactivity of these two systems induced by prolonged administration of these compounds can be assumed to play a role in the enhancement of SIF. However, under our experimental conditions neither promethazine nor atropine have produced any significant modification of SIF. Thus, at present, we lack data which could help to clarify the mechanism of SIF enhancement produced by chronically given ADs. In spite of that, more and more data suggest that facilitation of SIF, observed after multiple-dose treatment with ADs, may be a common and characteristic property of these drugs. In the light of this, our results could indicate potential, antidepressant activity of (−)OX. It is noteworthy, that in a preliminary double-blind cross over study in hospitalized depressed patients with (±)OX and (−)OX antidepressant-like activity of (−)OX has been observed [4].

In our study, in addition to fighting, the jumping behavior of rats subjected to foot-shock stimulation has been also recorded. Significant and marked increase in the frequency of jumping after single and multiple-dose treatment with (±)OX and (+)OX, strongly suggests that this effect can be to a great extent related to NA inhibiting properties of used drugs. As mentioned before, (±)OX is highly selective and potent NA-uptake inhibitor. Its NA-uptake inhibiting action is stereospecific and entirely confined to the (+)OX [21]. The involvement of NA in jumping behavior is also supported by recent observations [15] that α-adrenergic stimulation by clonidine increases the footshock induced jumping in rats.

Finally, an interesting observation in our study was that rats subjected to reptitive handling and manipulations show little response to footshock stimulation by comparison to nonhandled animals. Although it is generally recognized that repetitive handling may modulate behavioral responses there are little data on the particularity or mechanisms of such changes. This phenomenon requires further investigation. At present our findings once again emphasize the necessity and importance of matched and identically handled controls in chronic animal experiments.

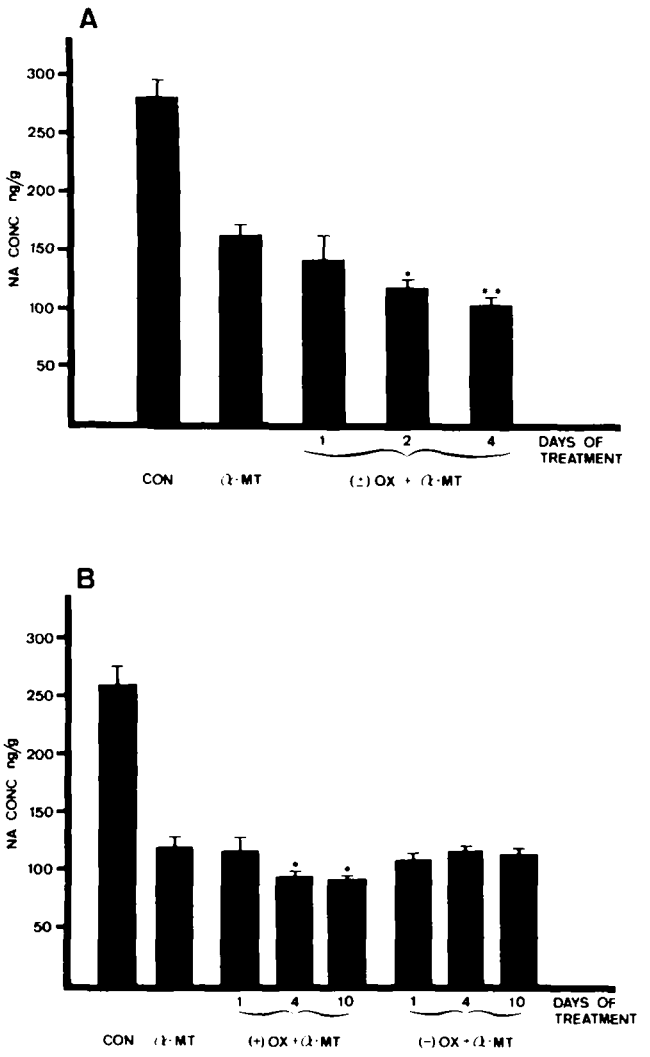

FIG. 1. NA-disappearance induced by α-MT in rats' cortex after treatment with (±)OX and its enantiomers. Two separate experiments were carried out. In Experiment A, groups of 5 rats received 10 mg/kg PO (±)-oxaprotiline once or once daily for 2 or 4 days. In Experiment B, the animals were treated with 5 mg/kg PO of either (+) or (−)-oxaprotiline once or once daily for 4 or 10 days. Two hours after the last treatment, they were given 250 mg/kg IP α-methyl-p-tyrosine methyester HCl (α-MT) and decapitated 4 hours later. NA concentrations were determined in the cortex. Data represent means ± S.E.M. in ng/g tissue; statistical calculations were done by means of Dunnett's t-test. *$p < 0.05$, **$p < 0.01$ vs. α-MT alone.

In conclusion; the results of the present investigation demonstrate an enhancement of SIF after multiple-dose treatment with MAP, (±)OX and NA-uptake active as well as NA-uptake inactive enantiomers of (±)OX. The mechanism responsible for this enhancement is unclear and needs further investigation. Strong facilitation of shock-induced jumping behavior by (±)OX and (+)OX seems, however, to reflect the NA-uptake inhibiting properties of drugs.

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