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Inhibition of Monoamine Oxidase by Viloxazine in Rats

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Dedicated to Professor Dr. Herbert Oelschläger on the occasion of his 65th birthday

Summary: *Biochemical and pharmacological investigations about the effect of the antidepressant drug viloxazine (Vivalan®) on catecholamine metabolism in rats led to the following results:*

1. Viloxazine exerts a dose and time dependent inhibition of monoamine oxidase activity of brain and liver mitochondrial fraction and tissue homogenates of hypothalamus, heart, liver, and adrenal glands, both in vitro and after oral and parenteral administration in vivo.

2. Consequently, an increase in catecholamine concentrations in brain of rats could be observed after pretreatment with viloxazine. In addition brain serotonin concentrations rose and 5-hydroxy-indoleacetic acid was diminished.

3. However, characterization of inhibition of monoamine oxidase activity by viloxazine in vitro revealed: Compared to the specific inhibitors clorgyline for MAO-A- and pargyline for MAO-B-activity, viloxazine was a very weak inhibitor both for MAO-A and MAO-B in vitro. The type of inhibition was competitive and reversible.

4. From the presented results and the results obtained by other laboratories it is concluded that inhibition of monoamine oxidase activity by viloxazine, although clearly demonstrated in animal experiments, may not be the only mechanism for an antidepressant action of the drug in man.

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Zusammenfassung: *Hemmung der Monoaminoxidase durch Viloxazin bei Ratten*

Biochemische und pharmakologische Untersuchungen über die Wirkung des Antidepressivums Viloxazin (Vivalan®) auf den Katecholamin-Stoffwechsel von Ratten führten zu folgenden Ergebnissen:

1. Viloxazin verursachte eine dosis- und zeitabhängige Hemmung der Aktivität der Monoaminoxydase der Mitochondrienfraktion von Gehirn und Leber sowie in Gewebehomogenaten des Hypothalamus, des Herzens, der Leber und der Nebennieren von Ratten, sowohl in vitro, als auch in vivo, nach oraler oder parentaler Applikation des Pharmakons.

2. Als Folge dieser Hemmung der Monoaminoxydase steigen die Konzentrationen von Katecholaminen und von Serotonin im Gehirn an, während die 5-Hydroxyindolessigsäure, das desaminierte Produkt von Serotonin, signifikant vermindert wurde.

3. Die Charakterisierung des Hemmtyps von Viloxazin in vitro ergab eine kompetitive und reversible Art der Hemmung. Viloxazin wurde darüber hinaus als schwacher Hemmstoff der Monoaminoxydase A und B erkannt bei Vergleichsuntersuchungen mit Clorgylin als Hemmstoff der A-Form der Monoaminoxydase und Pargylin als Hemmstoff der B-Form der Monoaminoxydase.

4. Die vorliegenden Ergebnisse und die Untersuchungen in anderen Laboratorien erlauben jedoch die Schlußfolgerung, daß trotz nachgewiesener Hemmung der Monoaminoxydase durch Viloxazin im Tierexperiment noch andere Mechanismen zur antidepressiven Wirkung des Pharmakons beim Menschen beitragen.

Key words: *Antidepressants · Monoamine oxidase · Viloxazine, pharmacology · Vivalan®*

1. Introduction

It is generally accepted that clinically used antidepressant drugs may exert their pharmacological effects mainly by inhibition of the reuptake of noradrenaline and serotonin (tricyclic antidepressant drugs) or by inhibition of monoamine oxidase (MAO-inhibitors), thereby increasing the postsynaptic concentration of these amines and consequently potentiating their transmitter functions. These prominent pharmacological activities of antidepressant drugs are probably related to their therapeutic actions [2, 4, 13]. In recent years it was observed that persistent high postsynaptic concentrations of noradrenaline or serotonin can lead to a down regulation of β-adrenergic receptors or serotoninergic receptors (for review see [12]). In addition a blockade of alpha-adrenergic receptors or histamine receptors by antidepressant drugs has been described [6, 15]. Concerning the mechanisms of recently introduced antidepressant drugs it is of interest to classify such drugs within the scheme to the current hypothesis of their mode of action, despite several objections against a close correlation between observed biochemical effects and their therapeutic action in patients.

Therefore the aim of the present paper was to characterize some pharmacological effects of viloxazine (2-(2-ethoxyphenoxymethyl)-2,3,5,6-tetrahydro-1,4-oxazine, Vivalan®[2]) in experiments in rats, especially its effect of monoamine oxidase activity, characterizing its mode of inhibition both in vivo and in vitro.

2. Methods

Male Sprague-Dawley rats (SIV 50) from Dr. Ivanovas, Kisslegg (FR Germany), body weight about 150 g, were used. The animals were housed in plastic cages (Makrolon®) in an air-conditioned, light-dark-cycled (12 h) room, with lights on from 6 a.m. to 6 p.m., and given food (Altromin®, standard diet; Altromin, Lage/Lippe, FR Germany) and tap water ad libitum. They received viloxazine in various doses orally (stomach tube) and parenterally as indicated under results. Controls were given 0.9% NaCl. Assay of monoamine oxidase was performed radiometrically according to Otsuka and Kobayashi [12] using various substrates as described under results. In some experiments we used preparations of mitochondria from rat brain [11] or rat liver [14] for assay of monoamine oxidase activity. Protein was determined according to the method of Bradford [3].

For determination of catecholamines (noradrenaline, dopamine) and serotonin we used high pressure liquid chromatography [1].

Data were statistically evaluated, using unpaired Student's t-test. Results are expressed as mean ± S.E.M.

[2]) Manufacturer: ICI-Pharma, Plankstadt (Federal Republic of Germany).

3. Results

Subcutaneous injection of viloxazine (70 mg/kg) caused a time dependent inhibition of monoamine oxidase activity in preparations of mitochondria from rat brain (Fig. 1). Using serotonin or β-phenylethylamine as substrates the maximum of inhibition occurred between 1 and 3 h after administration of the drug. 7 h after injection of viloxazine the monoamine oxidase activity was normal when compared to control preparations.

Concomitantly there was a significant increase in the concentration of brain dopamine and noradrenaline (Fig. 2). The maximum of transmitter concentrations run parallel with the inhibition of monoamine oxidase activity. Investigating increasing doses of viloxazine (35, 70, 105 mg/kg s.c.) 1 h after administration revealed in a dose dependent inhibi-

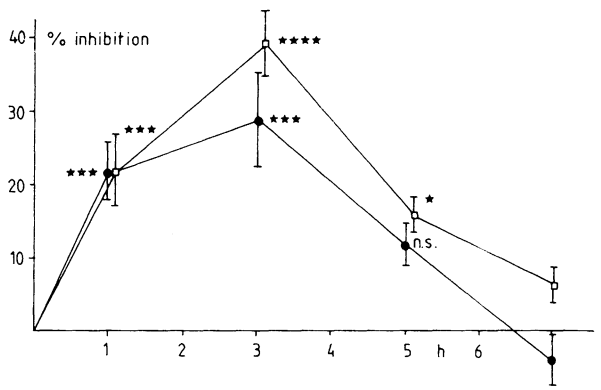

Fig. 1: Inhibition of monoamine oxidase in mitochondrial fraction of hypothalamus of rats after s.c. injection of 70 mg/kg viloxazine. Substrates: ^{3}H-serotonin = 1×10^{-8} mol/l; ^{14}C-β-phenylethylamine = 1×10^{-5} mol/l.

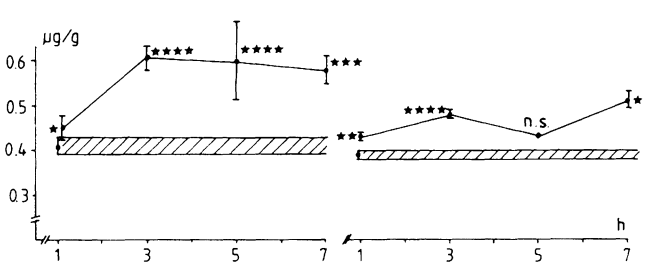

Fig. 2: Increase of catecholamine concentrations in rat brain after s.c. injection of 70 mg/kg viloxazine groups of 10 rats. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$. **Left:** dopamine; **right:** noradrenaline.

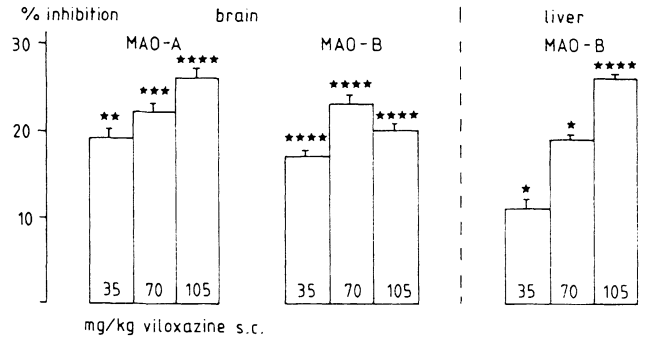

Fig. 3: Inhibition of the two forms of monoamine oxidase in rat brain and liver mitochondrial fraction 1 h after s.c. injection of increasing doses of viloxazine. Substrates: MAO-A = ^{3}H-serotonin 10^{-8} mol/l, MAO-B = brain: 10^{-6} mol/l, liver 10^{-5} mol/l, ^{14}C-β-phenylethylamine. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.

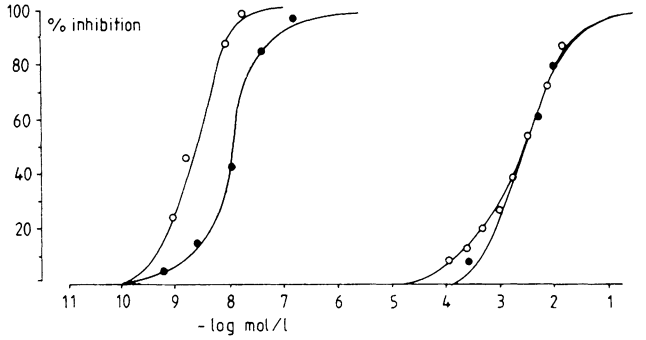

Fig. 4: Dose-response curves for inhibition of monoamine oxidase from rat brain homogenates by specific inhibitors of MAO-A or MAO-B in comparison to viloxazine. Incubation: 20 min, 37 °C. **Left:** ○, clorgyline, substrate: ^{3}H-serotonin 10^{-4} mol/l; ●, pargyline, substrate: ^{14}C-phenylethylamine 10^{-6} mol/l. **Right:** viloxazine, substrate: ○, ^{3}H-serotonin 10^{-4} mol/l; ●, ^{14}C-phenylethylamine 10^{-6} mol/l.

tion of monoamine oxidase in mitochondria of rat liver and hypothalamus using serotonin or β-phenylethylamine as substrates (Fig. 3).

The effect of viloxazine after repeated administration (35 mg/kg i.p. daily during 6 days) on monoamine oxidase activity (MAO activity) in various organs of the rat is depicted in Table 1. The inhibition of MAO was most pronounced in the rat heart, but also a significant inhibition of the enzymes could be observed in hypothalamus, liver, and adrenal gland. In these experiments tryptamine was used as a substrate for both forms of the enzyme MAO-A and MAO-B. In additional experiments we prepared fractions of mitochondria from hypothalamus and liver after chronic treatment with viloxazine (35 mg/kg s.c. daily over a period of 8 days). In the brain MAO-A activity was inhibited significantly by 14%, MAO-B activity by 23% and in liver MAO-B activity by 9%. The substrates used for MAO-A was ^{3}H-serotonin (10^{-8} mol/l) and for MAO-B was ^{14}C-phenylethylamin (10^{-6} mol/l).

Table 1: Inhibition of monoamine oxidase activity in rat tissue after s.c. injection of 35 mg/kg viloxazine daily during 6 days.

	Controls	n	Viloxazine	n	Inhibition (%)	p
Hypothalamus	1624 ± 26	5	1264 ± 43	5	22	****
Heart	1853 ± 106	5	1164 ± 82	5	37	****
Liver	1292 ± 35	6	1028 ± 32	5	20	****
Adrenal gland	1712 ± 27	4	1266 ± 75	5	26	***

Substrate: ^{14}C-tryptamine 10^{-5} mol/l; $\bar{x} \pm S_{\bar{x}}$ = cpm/min; n = number of rats; p = level of significance: *** $p < 0.005$, **** $p < 0.001$.

Analysing the results obtained with serotonin as a substrat in hypothalamus mitochondrial fraction according to Lineweaver-Burk we observed a mixed type of inhibition of monoamine oxidase activity by viloxazine (Fig. 4).

As a consequence of significant inhibition of monoamine oxidase activity in the brain we found increased concentrations of the putative transmitters, dopamine, noradrenaline and serotonin after acute (Fig. 2), as well as after chronic administration. The effect of viloxazine on noradrenaline and dopamine levels was dose dependent and more marked on dopamine concentrations (Table 2). Also serotonin concentrations in brain rose significantly and dose dependent after s.c. injection of viloxazine.

Chronic oral administration of increasing doses of viloxazine also caused an increase in brain serotonin concentrations and a decrease in 5-hydroxyindoleacetic acid (Table 3).

Table 3: Changes in serotonin and 5-hydroxyindoleacetic acid concentrations in brain of rats after oral administration of viloxazine daily over a period of 14 days.

Dose (mg/kg)	Serotonin			5-Hydroxyindoleacetic acid		
	Conc. (μg/g)	n	p	Conc. (μg/g)	n	p
Controls	0.40 ± 0.01	10		0.49 ± 0.01	9	
35	0.58 ± 0.04	9	****	0.41 ± 0.01	9	****
70	0.74 ± 0.06	10	****	0.37 ± 0.01	10	****

n = number of rats; **** $p < 0.005$; $\bar{x} \pm S_{\bar{x}}$.

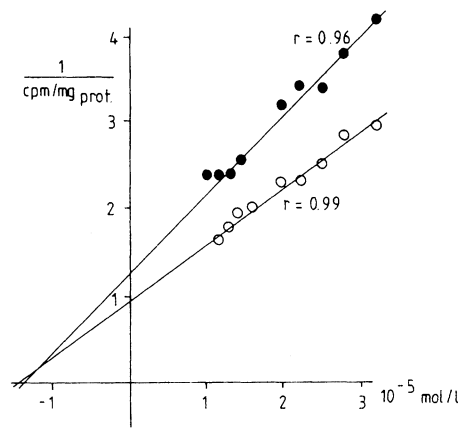

Fig. 5: Characterization of type of inhibition of monoamineoxidase by viloxazine. Lineweaver-Burk plot. Substrates: ^{3}H-serotonin: $1 \times 10^{-5} - 3 \times 10^{-5}$ mol/l. On mitochondrial fraction of hypothalamus of pretreated animals 35 mg/kg s.c. daily over a period of 8 days. ● = controls.

Table 2: Elevation in catecholamines and serotonin concentrations in brain of rats 1 h after s.c. injection after increasing doses of viloxazine.

Dose (mg/kg)	Dopamine			Noradrenaline			Serotonin		
	Conc. (μg/g)	n	p	Conc. (μg/g)	n	p	Conc. (μg/g)	n	p
Controls	0.45 ± 0.02	10		0.39 ± 0.01	10		0.46 ± 0.04	10	
35	0.53 ± 0.03	10	(*)	0.43 ± 0.01	10	****	0.63 ± 0.07	10	*
70	0.53 ± 0.03	10	*	0.43 ± 0.01	10	**	0.66 ± 0.05	10	**
105	0.59 ± 0.02	10	**	0.46 ± 0.01	9	****	0.72 ± 0.04	10	****

n = groups of 9–10 rats; (*) $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$; $\bar{x} \pm S_{\bar{x}}$.

Characterization of inhibition of the monoamine oxidase activity by viloxazine in vitro led to the following results:

1. Compared to the specific inhibitors clorgyline for MAO-A and pargyline for MAO-B, viloxazine was a very weak inhibition both for MAO-A and MAO-B (Fig. 5).
2. The type of inhibition under the in vitro conditions was competitive according to Lineweaver-Burk using serotonin as a substrate and a mitochondrial fraction of hypothalamus (Fig. 6).

   In further experiments it could be demonstrated that the inhibition in vitro under the same conditions as described in legend of Fig. 6 was reversible [9].

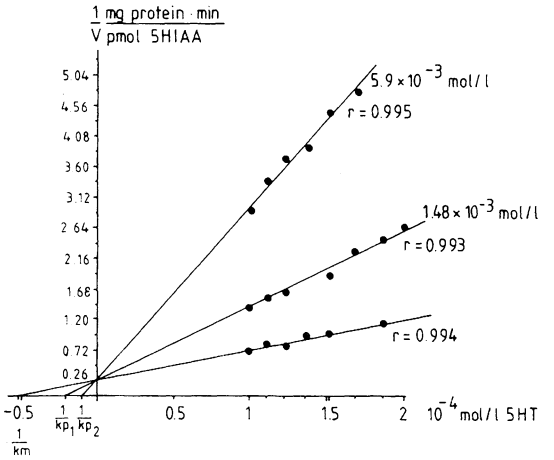

Fig. 6: In vitro characterization of inhibition of monoamine oxidase in mitochondrial fraction of rat hypothalamus by viloxazine. $K = 9.7 \times 10^{-5}$ mol/l; $K = 1.8 \times 10^{-4}$ mol/l.

4. Discussion

The results presented in this paper are in agreement with the assumption that viloxazine is capable to inhibit the monoamine oxydase activity of rat tissue in a dose dependent manner, after acute or chronic oral or parenteral administration of the drug. Moreover, the maximum of the inhibitory effect on monoamine oxidase activity runs parallel with an increase in rat brain catecholamines (dopamine, noradrenaline) and serotonin. These results are in disagreement with the conclusion of Lippman and Pugsley [10] that viloxazine does not inhibit monoamine oxidase in mice. However, these authors observed a potentiation of the L-dopa behavioral syndrome in the mouse by viloxazine and also an antagonism against reserpine induced ptosis and hypothermia in the same species. Since viloxazine also exerts some effects on uptake of catecholamines, like imipramine, the authors concluded, that uptake inhibition might be the reason for the observed drug action. In addition Greenwood [9] denied a significant inhibition by viloxazine on monoamine inhibitory oxidase activity, although he used only tyramine as substrate in his in vitro experiments and interestingly in vivo he could observe a short lasting increase in brain catecholamines and serotonin after injection of a single dose of viloxazine. Moreover, Lippman and Pugsley [10] noticed a decrease in concentrations of deaminated metabolites from ^{3}H-noradrenaline in rat hypothalamus after administration of 20 mg/kg i.p. viloxazine. This is in agreement with the significant inhibition of monoamine oxidase after administration of viloxazine in a single dose or repeated doses, not only in brain, but also in other organs reported under results. We could also demonstrate that viloxazine in vivo (Fig. 1/Fig. 3) and in vitro (Fig. 5) can inhibit monoamine oxidase in the A and B form. In addition the in vitro characterization of MAO-inhibition by viloxazine resulted in a competitive and reversible type of inhibition (Fig. 6). However, in vivo we observed a mixed type of inhibition of monoamine oxidase by viloxazine after chronic administration (Fig. 4).

This difference might be due to the formation of an active metabolite of viloxazine, which could shift the type of inhibition observed in vitro. In agreement with this assumption we observed a biphasic inhibition of liver monoamine oxidase occurring with two peaks of inhibition 1 and 5 h after 70 mg/kg s.c. viloxazine in a single dose [11].

Despite the high concentration necessary for viloxazine to inhibit the monoamine oxidase completely in vitro, when compared to clorgyline (MAO-A) and pargyline (MAO-B, Fig. 5), and the high doses used in vitro, it is likely that inhibition of monoamine oxidase of both forms in vivo makes a contribution to the antidepressant effect of the drug in patients. In line with this hypothesis a long lasting half life of viloxazine was reported in the liquor cerebrospinalis of depressive patients treated with viloxazine [5].

In addition a significant decrease in deaminated metabolites of noradrenaline and dopamine in urine of healthy volunteers after application of viloxazine was reported by Goodwin et al. [7] (see also [4]). Since monoamine oxidase in human brain might exist in form A and B, our results are demonstrating an inhibition of both forms by viloxazine could be of interest for the therapeutic action of the drug in man.

5. References

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